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A.S. Ginzburg

FERTILIZATION IN FISHES AND THE PROBLEM OF POLYSPERMY

Translated from Russian

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FERTILIZATION IN FISHES AND THE PROBLEM OF POLYSPERMY

(Oplodotvorenie u ryb i problema polispermii)

Edited by T. A. Detlaf

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PREFACE

[illegible] fertilization [illegible]

[illegible] of the fertilized egg and the [illegible]

Plants [illegible]

This book is the first extensive survey on fertilization patterns in relation to the problem of polyspermy especially with respect to fishes, which for many years have been the object of experimental embryological and cytological research of the author. The book contains detailed descriptions of the organization and properties of fish eggs and spermatozoa, their interaction and changes during fertilization. On the basis of published data and the author's own research, a mechanism for the protection of the egg from penetration by supernumerary spermatozoa is suggested in the case of various animals; ideas are also expressed as to the factors determining change in type of fertilization (physiological monospermy and polyspermy) in evolution. A supplement, useful for fish breeders, contains practical conclusions based on data presented.

This book is recommended to a wide circle of biologists, embryologists, cytologists, ichthyologists, students of theoretical and practical fish breeding.

There are 38 tables, 82 illustrations, and a bibliography containing 1,328 items.

PREFACE

Fertilization — the process initiating individual development — has long interested embryologists. During the taxonomic studies of various invertebrates and vertebrates, carried out in the late 19th century by O. Hertwig, Van Beneden, Fol, Boveri and others, a thorough cytological investigation of this process was made, so that it was possible to clarify the nature of meiosis and other phenomena observed in relation to fertilization. In the 20th century emphasis has been placed on the experimental study of fertilization pioneered by O. Hertwig, J. Loeb and F. R. Lillie, while the comparative embryological approach lost its former popularity. The favorite object of research was the sea urchin egg, which is excellent experimental material. Although considerable information accumulated on the changes in the fertilized egg (mainly in echinoderms), much remained unclear concerning the fertilization process, particularly questions related to polyspermy. A large number of spermatozoa surround the egg during fertilization but only one participates in karyogamy, whereas the others are either prevented from penetrating the egg (physiological monospermy) or from developing (physiological polyspermy) if they penetrate the cytoplasm. The mechanisms involved are not yet clear, nor are the factors determining which type of fertilization occurs in different animals. I believe that the difficulties in working out the problem of polyspermy are largely due to the lack of a broad, comparative embryological approach which could facilitate the discovery of general patterns missed in a study of a limited range of specimens. Since this is one of the main problems dealt with in this book, I shall endeavor to review as far as possible all the information on metazoans in the chapters devoted to the number of spermatozoa penetrating the egg at fertilization, the fate of supernumerary spermatozoa, the cortical reaction of the fertilized egg and the mechanism blocking polyspermy.

Fishes are exceptionally suitable for studying patterns of fertilization, since this vertebrate class displays great variety of organization of gametes and conditions of their interaction. Fishes' eggs differ greatly in size and in the amount and distribution of yolk. In some fishes, eggs are covered with delicate membranes penetrable by the spermatozoa, while in other fishes the eggs are covered by dense, sperm-impermeable membranes furnished with one or several micropylar canals. In some fishes, the gametic association takes place in the outer medium, in others it occurs in the genital tract of the female. Along with this manifold organization expressed in fishes are found both types of animal fertilization, namely, physiological monospermy and polyspermy.

The research on fishes also provides results not merely of theoretical interest, but of undoubted practical importance. Since many fishes are cultured, knowledge of their sexual products and fertilization patterns is necessary for the biotechnics of obtaining and preserving eggs and sperm and for rational methods of artificial fertilization.

Information published on the organization and features of gametes, their interaction and development of the fertilized egg in different fishes is limited and unrelated, and no reviews of the subject are available except for the short reviews devoted to fertilization in fishes (Rothschild, 1958; T. Yamamoto, 1961), which encompass only part of the available data and relate almost exclusively to a few teleosts.

In the present book, an attempt has been made to review the subject generally, using published data and my original results. Due to limitation of space, it was impossible to examine all aspects of the fertilization process. In particular, the patterns of formation, transfer and association of pronuclei and the development of bilateral symmetry, which have independent significance and are less closely connected to polyspermy, have not been elucidated here.

Conclusions significant for fish breeding are given in the supplement.

The original research presented here was carried out at the Filatov Experimental Embryology Laboratory of the Severtsov Institute of Animal Morphology (incorporated in 1967 in the Institute of Development Biology) of the Academy of Sciences of the USSR. I wish to extend sincere thanks to its director B. L. Astaurov for his constant support and assistance in the course of the research.

The constant contact with T. A. Detlaf, her lively interest in the essentials of the work and the heated discussions of results of the research were invaluable, as well as her observations concerning the manuscript. L. A. Filatova aided greatly in experiments and analysis of results. Friendly assistance was repeatedly given by my colleagues T. B. Aizenshtadt, S. G. Vasetskii, S. I. Davydova, A. I. Zotin, S. E. Zubova, M. N. Skoblina and E. V. Chulitskaya, and in the electron microscope research by coworker I. I. Glezer of the Psychiatric Institute of the Academy of Medical Sciences of the USSR. To all these scientists, I wish to express my deep gratitude.

I also wish to thank I. P. Grechanovskii, V. A. Skorichenko, and V. I. Yankovskaya for their help in obtaining experimental material during work at the Rogozhkin sturgeon breeding station (1957–1961) and at the Svirsk fish breeding station (1959–1961).

Chapter 1

THE EGG

The egg cell fulfills a special function quite different from that of any other cell — that of giving rise to a new individual. To fulfill this function, wide potencies must be preserved and nutrients must be accumulated for the embryo to meet its plastic and energic requirements until it can obtain exogenous food. During oogenetic differentiation of the female gamete, reserve substances (mainly in the form of yolk granules and lipid inclusions) accumulate in the cell. By the end of oogenesis, all metabolic processes in the egg are retarded, the nucleus consolidates at a particular stage of meiosis (differing in different animals), and the egg is prepared for penetration of the spermatozoon to begin embryonic development.

The description of the mature fish egg commences with the examination of the main features of its structure, often similar not only in various groups of fishes but also among different classes and even different phyla. Since the distinctive structure of the egg is formed during oogenesis, this period will also be discussed (although a full examination is beyond the scope of this book). Specific features of egg structure characterizing various groups of fishes will be described separately in the following section. Features of the cavity fluid in which ovulated eggs are immersed and, finally, the egg's fertility and its preservation under various conditions will be discussed.

1.1. ORGANIZATION AND CHEMICAL COMPOSITION OF THE EGG

1.1.1. **Nucleus**

Stage of inhibition of meiosis in fishes and other animals. Inhibition of nucleic changes in the maturing egg occurs in different animals at different stages of meiosis: before commencement of meiotic division (flatworms and roundworms, some segmented worms, many mollusks, crustaceans), at the metaphase of the first division (planaria, ribbon worms, some segmented worms and mollusks, insects, enteropneusts), at the metaphase of the second division (almost all chordates, some crustaceans) and after completion of meiotic division at the stage of pronuclear formation (some coelenterates, sea lilies, sea urchins, ascidians).

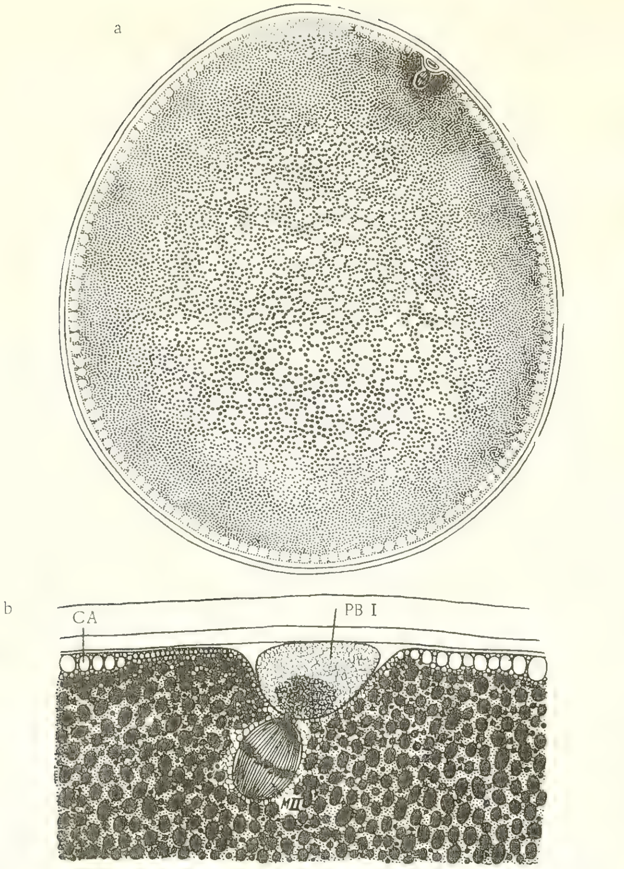

FIGURE 1. Mature unfertilized egg of Lampetra fluviatilis:

a — section of egg along animal-vegetal axis at low magnification; b — region of egg nucleus; c — part of lateral surface of egg at high magnification (after Herfort, 1901). YG — yolk granule; CA — cortical alveoli; M II — metaphase of second maturation division; PB I — first polar body; zr — zona radiata.

In cyclostomes and fishes, as in most other chordates, meiosis is blocked at the metaphase stage of the second maturation division (Figure 1, a, b). Hoffman (1881), clearly described the spindle at the metaphase stage in mature eggs of teleosts (Scorpaena porcus, S. scrofa, Julis vulgaris) and indicated that soon after insemination the polar body is divided off simultaneously with formation of the female pronucleus. Subsequently, the blocking of meiosis at metaphase II was established for many kinds of fishes: Lampetra (Petromyson) fluviatilis (Herfort, 1893, 1901; Chubareva, 1957 a,b), Pristiurus melanostomus and also Torpedo (Kashchenko, 1890; Rückert, 1891b, 1892a), sturgeons — Huso huso, Acipenser güldenstädti, A. stellatus, A. ruthenus (Mansurova, 1951; Faleeva, 1953; Kazanskii, 1954, 1957; Persov, 1954b; Ginzburg, 1957a, 1959), and teleosts — Clupea harengus pallasi (Kanoh, 1953), Oncorhynchus keta (K. Yamamoto, 1952a), Coregonus albula, Abramis brama (Arndt, 1960b), Megalobrama terminalis (Tchou-Su et al., 1960; Tchou-Su and Wang Yu-lan, 1962), the bisexual form of Carassius auratus gibelio (Cherfas, 1966) and Carassius auratus (Tchou-Su and Chen-Chao-hsi, 1936; Tchou-Su and Wang Yu-lan, 1962), Cyprinus carpio (Ozima, 1943), Cobitis biwae (Kawamura and Motonaga, 1950) and Misgurnus fossilis (Belyaeva and Cherfas, 1965). In Vimba vimba fertilization takes place somewhat later, at the early or complete anaphase II (Sakun, 1955).

The indications in the literature that in certain fishes (Ctenolabrus adspersus) and various salmon species (Salmo trutta, S. gairdneri = S. irideus) (Boehm, 1892; Blanc, 1894; Behrens, 1898), and S. ischchan (Negonovskaya, 1960) the egg is fertilized already at completion of the first meiotic division at metaphase I are based on incomplete and incorrectly interpreted data and should be regarded as erroneous. In recent research on nuclear changes in the fertilized egg of S. ischchan (Negonovskaya, 1966), it was found that, as in other fishes, the mitotic figure of the mature egg is metaphase II.

A special case is that of egg maturation in the unisexual form of Carassius auratus which reproduces gynogenetically: the egg is stimulated to develop by spermatozoa of another species (goldfish or carp) while the spermatozoan nucleus does not unite with the egg nucleus and is eliminated. As can be assumed (Golovinskaya, 1954), only one polar body is divided off in this form. The entire meiotic process is sharply different (Cherfas, 1966); the first maturation division is not completed but extends to the anaphase stage, after which the chromosomes once again join at the equatorial plate. At this stage, which provisionally may be called the metaphase II stage, the egg ovulates.

Morphology of nuclear changes at meiosis in fish. The meiotic process was traced in detail in the sturgeon eggs (Kazanskii, 1957; Vasetskii, 1966, Detlaf and Davydova, 1968). Egg maturation was stimulated by administration of a hypophysis preparation, as is done at sturgeon breeding stations.

The sturgeon oocyte at the initial stage (stage IV of maturation, Figure 2,I) possesses a large bladderlike nucleus (germinal vesicle) displaced toward the animal pole; the nuclear membrane on the side toward the vegetal pole has a wavy contour.

The nucleoplasm of the germinal vesicle contains at this time numerous nucleoli, usually located adjacent to the nuclear membrane, (Detlaf and Davydova, 1968). There is also a "karyosphere" consisting of a small dense body which does not yet fully enclose the spiral prophasic chromosomes with nucleoli material.

After injection of the hypophysis preparation, the nuclear vesicle rises almost to the surface of the oocyte, nucleoli dissolve and disappear, and the folding of the nuclear membrane toward the vegetal pole increases with partial extension of the nucleoplasm into the cytoplasm (Figure 2, II). A large space filled with yolk-free material is formed at this site (Figure 2, III). As the nuclear membrane disintegrates, the germinal vesicle diminishes rapidly in size and the nucleoplasm mixes with the cytoplasm (Figure 2, IV—VIII), but not completely since the cytoplasm around the animal pole of the mature egg still contains small islets of nucleoplasm forming a ramified network (not shown in Figure 2, see page 64 and Figure 12). As the nuclear membrane dissolves, the spindle of the first meiotic division forms near the "karyosphere" with small spiral chromosomes randomly distributed on it. As the achromatic figure moves toward the animal pole, the chromosomes become arranged on the equatorial plane of the spindle, forming a metaphasic plate. The spindle is oriented perpendicular to the oocyte surface (Figure 2, VIII). With pinching off of the first polar body, the spindle of the second meiotic division appears at metaphase II; meiosis is halted and the egg, having reached the stage of development necessary for fertilization, ovulates (Figure 2, IX).

The nuclear changes observed at meiosis in teleosts are similar in the main to those described above (Kawamura and Motonaga, 1950; Sakun, 1955, 1961a; Tchou-Su and Wang Yu-lan, 1962; Belyaeva and Cherfas, 1965; Latif, 1966, etc.). In fishes of this group, as in sturgeons, the egg reaches metaphase II before ovulation.

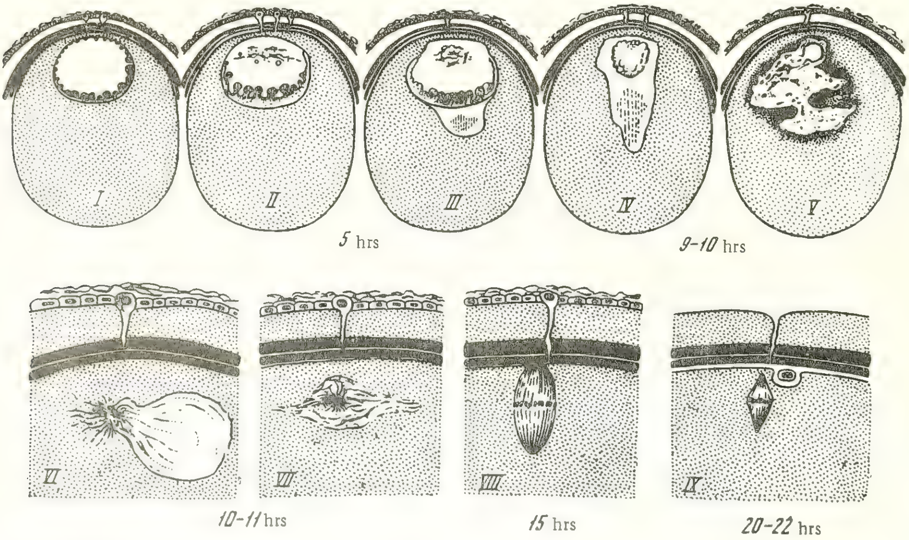

FIGURE 2. Diagram of maturation process of a sturgeon egg (according to Kazanskii, 1957) Temperature 17.3–20.8°C. See text for explanation .

In acipenserids (Kazanskii, 1957; Ginsburg, 1959) and teleosts (Hoffmann, 1881; Behrens, 1898; Tchou-Su and Chen-Chao-hsi, 1936; Sakun, 1955; Tchou-Su and Wang Yu-lan, 1962; Belyaeva and Cherfas, 1965), as well as in many other animals (Wilson, 1925), the achromatic figures of the meiotic divisions are of the anastral type, lacking centrioles and asters (see Figures 1 and 2). In sturgeon (Kazanskii, 1957) and loach (Belyaeva and Cherfas, 1965), the achromatic figure of metaphase II is 115 times smaller than the figure of the first division; a similar difference occurs in the androgynous form of goldfish, where the width of the spindle at the equatorial plane diminishes along with reduction in chromosome number (Cherfas, 1966). In vimba, the spindles of both divisions are of equal length (Sakun, 1955).

Duration of the meiotic process has been studied in considerable detail in the case of sturgeons and loach. The duration of development was studied in terms of the system elaborated by T. A. Detlaf (Detlaf and Detlaf, 1960, 1961) which allows for comparison of similar processes in the same species of various temperatures, as well as their duration in different species.

The essence of this method is that duration of development is expressed not in time units (hours, minutes) but in biological units, such as the duration of a particular developmental stage (measured at the same temperature as in the experimental conditions). A convenient unit, comparable in many animals, is the duration of the mitotic cycle at the time of synchronous cleavage divisions in the given species, which I shall call τ_0 (or Detlaf—Neifakh, 1961; Zotin, 1962, 1964). This unit already takes into account the specific developmental rates and their relation to external conditions; thus the actual conditions of the experiments are noted, as it were, and the general time relations clarified.

This method characterizing the duration of development was used to evaluate the duration of the entire maturation process in sturgeon eggs from injection of the hypophysis preparation to ovulation, as well as the duration of separate stages. It was shown that in Acipenser güldenstädti and A. stellatus the total duration of the maturation period is $^1/_5$ (0.19±0.02) that of the embryonic development from fertilization to hatching of the first larvae (Detlaf and Zubova, 1962) or 25—28 τ_0 (Detlaf and Zuichenko, 1963).

The meiotic prophase terminates in sturgeons at about the middle of the maturation period; during 13—14 τ_0 after the hypophysis injection, the germinal vesicle membrane disintegrates and the nucleus enters prometaphase of the first meiotic division (Detlaf and Davydova, 1968). Metaphase I in the oocytes of various female specimens of Acipenser güldenstädti and A. stellatus lasts 14.9—19.4 τ_0 (Detlaf and Zuichenko, 1963; Vasetskii, 1966). The duration of different meiotic phases in the sturgeon is as follows: metaphase I — 3.4, anaphase I—1.2, and telophase I together with prophase II — 1.2 τ_0 (Vasetskii, 1966).

In a study of meiotic divisions of oocytes in loach (Belyaeva and Cherfas, 1965) it was established that the transfer of the germinal vesicle to the animal pole lasts 50—70% of the time from gonadotropin injection until ovulation. In the next 20—30% of this period, the germinal vesicle dissolves, nucleoplasm disperses into the cytoplasm and nucleolic changes occur (fusion of nucleoli, formation of a sheath for prophasic chromosomes from nucleolar material and its destruction at prometaphase I). All subsequent phases up to metaphase II occupy 5—8% ($3\tau_0$) of the time period from injection to ovulation (as determined for loach by Ignat'eva and Kostomarova, 1966).

Correlation of the data obtained for sturgeons and loach shows that the time ratios between the individual stages in the maturation process in different fishes probably varies substantially. Thus the period from metaphase I to metaphase II in the sturgeon lasts about $6\tau_0$, while in the loach it lasts only about $3\tau_0$.

1.1.2. Cytoplasm

The fish oocyte cytoplasm contains the same basic elements as that of somatic cells: the basic substance (hyaloplasm), endoplasmic reticulum, Golgi material and mitochondria. In addition, in sturgeons and teleosts there are cortical bodies on the egg surface, and in sturgeons pigment granules in the subcortical layer. Finally, oocytes contain a very large amount of yolk inclusions.

Hyaloplasm and endoplasmic reticulum. Under a light microscope, the cytoplasm of somatic cells appears to have no structure. Electron microscopy reveals a dense system of vesicles and tubules, i.e., the endoplasmic network or reticulum enclosed in its basic substance (hyaloplasm) (see Porter, 1961). The endoplasmic reticulum is poorly developed in the oocytes of most animals (see Williams, 1965) and is represented by a few vesicles whose surfaces are smooth or strewn with ribosomes. However, in some species the membrane structure is highly developed. In the hyaloplasm of the oocytes a large number of free ribosomes and annulate lamellae are encountered regularly which closely resemble the nuclear membrane in structure and apparently originate from it.

Scarcely any data are available on the cytoplasmic ultrastructure of fish oocytes. In young oocytes of Xiphophorus helleri and Molliensisia sphenops the endoplasmic reticulum is represented by vesicles which communicate with the perinuclear cistern near the nucleus; the free ribosomes are arranged in rosettes (Porte and Zahnd, 1962). The same elements — vesicles and free ribosomes — are present in the hyaloplasm of the cortical region of sturgeon oocytes (see Plate XIa).

Golgi material. Cytological methods for detecting Golgi bodies have led to the detection of cytoplasmic regions capable of reducing silver nitrate and osmium tetroxide (Dalton, 1961). By silver and osmium treatment it is possible also to discover in the oocytes of various animals regions shaped like curved plates and rods (dictyosomes), granules, vesicles and filaments, which anastomose and form networks. Studies of the transformation of Golgi material at oogenesis in animals of remotely related groups reveal a general pattern (Vainer, 1925; Srivastava, 1965): in oogonia and young oocytes this material is situated near the nucleus and, except for a number of invertebrates, is organized in a reticular apparatus formed of filaments and cords. In subsequent stages the Golgi material increases in volume; the reticular apparatus divides into saclike bodies dispersed throughout the cytoplasm. Later the Golgi bodies are concentrated in the subcortical cytoplasm. The timing of these processes may vary considerably in different animals.

The Golgi material undergoes similar changes at oogenesis in fishes (Wheeler, 1924; Narain, 1930, 1937; Nath and Nangia, 1931; Singh and Boyle, 1938; Gerbil'skii, 1939; Nath, 1957; Chopra, 1958a, b, 1960) and lampreys (Chubareva, 1957b). In young oocytes it is concentrated in a limited region near the nucleus; later it is dispersed through the cytoplasm and shifted toward the periphery of the oocyte. This process has been described differently in different experimental material. In young oocytes of Gasterosteus aculeatus, the Golgi material forms a compact mass next to the nucleus (Singh and Boyle, 1938); this mass later forms a network around the nucleus. In subsequent stages, the quantity of Golgi material increases, the network is shifted to the subcortical cytoplasm where it splits into small, rod-shaped bodies and irregular granules. In Ophiocephalus punctatus, on the other hand, the Golgi bodies in young oocytes are described as isolated falciform granules and lamellae (Narain, 1930; Nath and Nangia, 1931). This does not indicate the existence of real differences in the distribution of Golgi material in oocytes of the species studied, since patterns obtained with various methods of silver and osmium treatment and at different times even on a single object vary considerably (Hibbard and Parat, 1928). In particular, structures like anastomosing cords and septa might result from the deposition of silver and osmium at intervals between individual Golgi complexes.

The Golgi material in the oocytes of teleosts is often poorly exposed by osmium treatments. In the oocytes of plaice (Wheeler, 1924) and three-spined stickleback (Singh and Boyle, 1938) the Golgi bodies are well exposed by silver treatment, but when treated with osmium show irregular patterns; in the oocytes of Ophiocephalus, Barbus and Boleophthalmus they do not turn black when fixed in Champy's fluid, but become impregnated only after lengthy osmium treatment (Chopra, 1960).

The classical cytological studies provided information concerning the distribution of Golgi material (if critical comparisons are made of results obtained with various procedures), but did not allow for an idea of its structure, since its components are beyond the resolution of a light microscope.

Recent ultramicroscopic research on various objects has shown that in actively functioning cells (for example, glandular cells) the Golgi complex consists of 1) large vacuoles, 2) an adjacent system of flattened sacs which, after expanding, may turn into vacuoles, and 3) a group of small vesicles arising by means of budding at the ends of the flattened sacs.

Poor development of the Golgi complexes is characteristic for mature eggs of various animals and for a number of other cells with low metabolic activity (see, for example, Afzelius, 1956b; Wischnitzer, 1962, 1964). The ultrastructure of the Golgi material in fish oocytes has not been studied.

Mitochondria. The distribution of mitochondria in the egg cell changes considerably during oogenesis (see Srivastava, 1965). In young oocytes of teleosts (Hibbard and Parat, 1928; Narain, 1930, 1937; Nath and Nangia, 1931; Konopacka, 1935; Singh and Boyle, 1938; K. Yamamoto, 1956c; Nath, 1957; Chopra, 1958a, b, 1960) and lampreys (Chubareva, 1957b), the mitochondria, like the Golgi material, are distributed at first near the nucleus;

as their number increases, they are dispersed throughout the cytoplasm. It is possible that the mitochondria participate in formation of the yolk inclusions (see below) in some animals during vitellogenesis.

By the end of vitellogenesis, the mitochondria are usually discernible only in the yolk-free cortical layer of the cytoplasm. However, in oocytes of fishes whose yolk granules do not coalesce, mitochondria were also present in the cytoplasmic stroma of the yolk-rich part of the egg. In Boleophthalmus dussumerii (Gobioidei) there are large invididual mitochondria among the yolk granules at the end of vitellogenesis (Chopra, 1960). This is also shown by the following experiment performed on Misgurnus fossilis. Beginning at the fourth to fifth cleavage division, the blastodisc was removed by centrifugation of the embryo in a sucrose gradient (Kostomarova and Neifakh, 1964). The yolk was then homogenized and centrifuged. A mitochondria-rich fraction was separated; in the yolk-granule fraction, electron-diffraction patterns showed mitochondria scattered among the destroyed yolk inclusions (Aizenshtadt and Neifakh, personal communication). Some of the mitochondria in the fertilized loach egg are apparently closely associated with yolk granules; after repeated washing of light components from a homogenate of the loach egg, the respiration of the heavy fraction (containing yolk inclusions) showed 27% of the respiration of the entire homogenate (Abramova et al., 1965).

Mitochondria of oocytes of most animals studied have a typical ultrastructure; they consist of an inner folded membrane and a matrix. In the mature sturgeon egg (Ginzburg, unpublished) the surface layer of the cytoplasm contains many elongate and dumbbell-like mitochondria with a considerable number of folds perpendicular to the long axis (see Plate I, a, d).

Cortical granules and alveoli. In the cortical layer of mature eggs of animals of various phyla there are peculiar inclusions or bodies called cortical granules and alveoli. It will be shown later that these inclusions have an important function in fertilization, since they are involved in protecting the egg from penetration by supernumerary spermatozoa; special attention will therefore be paid to them. Here, we shall deal with the origin, structure and features of the cortical granules and alveoli in fish eggs, as well as briefly summarizing similar formations in the eggs of other animals. The first work published on this question was by Zotin (1961a).

In teleosts the cortical bodies consist of fluid-filled vesicles lying close to the egg surface, imparting to the cortical layer an alveolar structure. Accordingly, these structures were named cortical alveoli. In fixed eggs of chum salmon (Yamamoto, 1951b) and trout (Ginzburg, 1961a), the coagulated contents of the cortical alveoli occupy only a small portion of their original volume. On the other hand the contents of cortical granules of sturgeons are only slightly compressed on fixation and appear as compact formations.

Cortical granules have been found only relatively recently in all sturgeon species studied — Acipenser güldenstädti, A. stellatus, Huso huso (Detlaf, 1957, 1961b, 1962; Ginzburg, 1960b, 1961a).

The study of the cortical alveoli of teleosts has a longer history. The first investigators to observe cortical alveoli in teleost eggs were the Norwegian ichthyologists Boeck (1871) and Sars (1876). Boeck found a layer of spherical, strongly refractive bodies just below the membrane of Baltic herring eggs. Sars described numerous, variously sized droplets scattered unevenly over the whole yolk surface in cod eggs. Both investigators regarded these inclusions as oil droplets.

Kupffer (1878), who investigated unfertilized eggs of the Baltic herring, drew attention to the strongly light-refracting homogeneous globules, 0.008—0.020 mm in diameter, distributed at their surface. He called them "yolk grains" (die Dotterkörner) to distinguish them from the true yolk inclusions, which are called yolk spheres. Kupffer first established that the cortical alveoli disappear at fertilization, indicating that as the formation of the perivitelline space begins the "yolk grains" disappear, apparently dissolving.

Ryder (1884) described strongly light-refracting globules or vesicles in the cortical layer of an unfertilized cod egg and noted their disappearance at fertilization. His suggestion, later proven correct, was that the contents of the vesicles are discharged into the perivitelline space ("respiratory chamber" according to the terminology of Newport).

Next, cortical alveoli were observed in the eggs of Labridae — Ctenolabrus adspersus and Tautoga onitis (Agassiz and Whitman, 1885a, b) and Lucioperca (Stizostedion) vitreum (Reighard, 1893). In the 20th century, these bodies have been noted in most other teleost species belonging to 8 different orders (Table 1); therefore, cortical alveoli are a stable component of fish eggs.

Appearance of cortical granules and alveoli in fish oocytes. The formation of cortical granules in sturgeons has not been studied. It is only known that right up to dissolution of the germinal vesicle in the oocyte the cortical granules occupy a definite position in the cortical layer (Detlaf, 1961b, 1962b).

In teleosts, the precursors of cortical alveoli — polysaccharide-rich vacuoles — appear at the beginning of intensive development of the oocyte during which various inclusions characteristic for the mature egg are formed and accumulated.

The appearance of these vacuoles was first observed by Guthrie (1925, 1928) in Fundulus. Spek (1933), while studying the oogenesis of teleosts with vital dyes, described the appearance of numerous droplets filled with a basic colloid at the periphery of the oocyte. Later the appearance and accumulation of vacuoles was traced by Konopacka (1935, 1937) and many others.

The origin of the precursors of the cortical alveoli was studied cytologically in detail in oocytes of Liopsetta obscura and Clupea harengus pallasi (Yamamoto, 1955a, 1956c, f). It was shown that they arise near and, apparently, with the participation of argentophil bodies corresponding to the Golgi material.

In young oocytes of plaice and herring, small granules (easily distinguished by silver staining) appear in the perinuclear region. Later, these are dispersed throughout the cytoplasm, forming filaments which anastomose into a network. In subsequent stages the argentophil filaments shorten, become rod-shaped and move to the periphery of the oocyte. The morphology and distribution of the argentophil granules and filaments correspond to that of Golgi material in the oocytes of other animals. Despite this, Yamamoto regarded the argentophil bodies as formations of a different kind since they did not react to osmium treatment like classical Golgi bodies, showed no positive lipid reaction by Ciaccio' method, and resisted the effect of gum spirits (a solvent of fats). It should be noted in this connection that a portion of the argentophil bodies in herring is extractable in gum spirit

TABLE 1. Cortical granules and alveoli in eggs of various animals

Phylum, class, order	Species	Diameter (μ)	Chemical nature of contents	Author
Plathelminthes				
Turbellaria				
Polycladida	Notoplana humilis, etc. (11 species)			Kato, 1940
Nemathelminthes				
Nematoda				
Rhabditoidea	Rhabditis dolichura			Bütschli, 1873
	Rhabditis			Pal'chikova-Ostroumova, 1926
	Diplogaster longicauda			Ziegler, 1895
Ascaridoidea	Parascaris equorum			Van Beneden, 1883
	(Ascaris megalocephala)		Calcium phosphate, traces of protein	Fauré-Fremiet, 1943
			Inorganic phosphorus, calcium	Panijel, 1951
		To 10–15	Protein, inorganic matter	Makarov, 1953a, b, 1958
				Fauré-Fremiet et al., 1954
Annelidae				
Polychaeta				
Errantia	Nereis limbata			Lillie, 1911; 1919; Spek, 1930, Novikoff, 1939a, ; Costello, 1949, 1958
	Nereis Dumerilli			Spek, 1930
	Nereis japonica		Acid mucopolysaccharides	Taoka, 1956c
			Acid mucopolysaccharides, proteins	Sawada, 1960
	Nereis diversicolor			Pasteels, 1956
	Platynereis megalops			Just, 1915
	Tylorrhynchus heterochetus		Acid mucopolysaccharides	T. Yamamoto, 1952; Taoka, 1956a, b
	Lysidice viridis			Takashima et al., 1955; Katsura et al., 1955
	Glycera dibranchiata			Simpson, 1962
Sedentaria	Sabellaria alveolata	0.4×0.5–0.75		Pasteels, 1965b
	Chaetopterus pergamentaceus			Lillie, 1906; Rebhun — see Allen, 1958
Mollusca				
Bivalvia				
Filibranchia	Mytilus edulis			Reverberi and Mancuso, 1961
		0.8		Humphreys, 1962

Eulamellibranchia	Mactra sp.			Kostanecki, 1904
	Mactra veneriformis		Neutral mucopolysaccharides	Sawada and Murakami, 1959
		2.0		Sawada, 1964
	Spisula solidissima			Allen, 1953; Rebhun, 1962
	Barnea spinosa (b. candida)	0.4—0.6	Acid mucopolysaccharides	Pasteels and de Harven, 1962
	Gryphaea angulata		Acid mucopolysaccharides	Pasteels and Mulnard, 1957
Echinodermata				
Crinoidea				
Articulata	Comanthus japonicus			Dan and Dan, 1941
Holothuroidea				
Dendrochirota	Cucumaria frondosa			Lönning, 1967a
Asteroidea				
Phanerozonia	Asterina pectinifera			Motomura, 1941
Cryptozonia	Marthasterias glacialis			Lönning, 1967a
Echinoidea				
Cidaroida	Glyptocidaris crenularis			Osanai, 1960b
Centrechinoida	Arbacia punctulata			E. N. Harvey, 1911; McCulloch, 1952
		0.79		Moser, 1939
	Arbacia lixula (A. pustulosa)			Lindahl, 1932; Monné and Hårde, 1951; Lönning, 1967a
	Temnopleurus toreumaticus	1.0		Takashima, 1960
	Temnopleurus hardwickii		Acid mucopolysaccharides	Osanai, 1964
	Mespilia globulus			Endo, 1952
	Genocidaris maculata			Lönning, 1967a
	Echinus esculentus	0.79—0.89		Afzelius, 1956a
	Echinus acutus			Lönning, 1967a
	Psammechinus miliaris			Monné and Hårde, 1951
		0.71—0.77		Afzelius, 1956a
	Psammechinus microtuberculatus			Lönning, 1967a
	Lytechinus variegatus			Hendee, 1931
	Tripneustes gratilla			Balinsky, 1961
	Toxopneustes pileolus			Balinsky, 1961
	Paracentrotus (Strongylocentrotus) lividus			Runnström, 1928; Lönning, 1967a
			Acid mucopolysaccharides	Monné and Slautterback, 1950

TABLE 1 (Continued)

Phylum, class, order	Species	Diameter (μ)	Chemical nature of contents	Author
		0.60—0.75		Pasteels, 1965b
	Strongylocentrotus droebachiensis	0.92		Afzelius, 1956a; Lönning, 1967a
	Strongylocentrotus purpuratus	0.6—1.2		Allen, 1957
	Strongylocentrotus (Hemicentrotus) pulcherrimus	0.5		Motomura, 1941; Endo, 1952
		1.5—2.0		Takashima, 1960
			Acid mucopolysaccharides	Osanai, 1964
	Strongylocentrotus nudus		Acid mucopolysaccharides	Osanai, 1964
	Strongylocentrotus pallidus			Lönning, 1967a
	Pseudocentrotus depressus			Endo, 1952
	Heliocidaris (Anthocidaris) crassispina			Endo, 1952
	Sphaerechinus granularis			Lönning, 1967a
Exocycloida	Clypeaster japonicus	0.5—1.3		Endo, 1952
	Peronella (Laganum) lesueuri			Endo, 1952
	Echinocyamus minutus (E. pusillus)			Lönning, 1967a
	Brissopsis lyrifera		Proteins, acid mucopolyssacharides	Monné and Hårde, 1951
		To 0.8		Lönning, 1963, 1967a
	Spatangus purpureus	0.90		Afzelius, 1956a
	Echinocardium cordatum		Proteins, acid mucopolysaccharides	Monné and Hårde, 1951
		0.50		Afzelius, 1956a
				Lönning, 1967a
	Echinocardium pennatifidum			Lönning, 1967a
	Echinocardium flavescens			Lönning, 1967a
Chordata				
Enteropneusta	Saccoglossus kowalevskii			Colwin and Colwin, 1954a; Colwin et al., 1956
Acrania	Branchiostoma (Amphioxus) lanceolatus			O. Van der Stricht, 1895; Sobotta, 1897
	Branchiostoma caribaeum			Guraya, 1967

Cyclostomi				
Petromyzoniformes	Lampetra fluviatilis	To 12		Herfort, 1893, 1901
	Lampetra planeri			Kille, 1960a
	Lampetra reissneri (L. planeri Regan)	7–13		T. Yamamoto, 1944c
			Neutral mucopolysaccharides	Kusa, 1957a
	Lampetra japonica		Neutral mucopolysaccharides	Kusa, 1957a
	Lampetra wilderi			Okkelberg, 1914
Pisces				
Acipenseriformes	Huso huso		Acid mucopolysaccharides associated with proteins	Detlaf, 1957, 1961b, 1962
	Acipenser güldenstädti colchicus	2.2x3.8		
		0.2–4.0		Ginzburg, unpublished
	Acipenser stellatus	2.2x3.8	Acid mucopolysaccharides associated with proteins	Detlaf, 1957, 1961b, 1962
		0.2–3.2		Ginzburg, unpublished
Clupeiformes	Clupea harengus membras	8–20		Kupffer, 1878
				Kryzhanovskii, 1956
	Clupea harengus pallasi			Kanoh, 1952a
		1.0–37.5		Kanoh, 1953
			Neutral mucopolysaccharides, proteins	K. Yamamoto, 1955a, 1956d, 1958b
			Polysaccharides, proteins	T. S. Yamamoto, 1957
	Caspialosa kessleri pontica			Kryzhanovskii, 1956
	Oncorhynchus keta			Kanoh, 1950
		12–25		K. Yamamoto, 1951b
		3–30	Acid mucopolysaccharides, few proteins	Kusa, 1954, 1956
	Oncorhynchus nerka		Acid mucopolysaccharides, few proteins	Kusa, 1954, 1956
	Salmo trutta m. lacustris			Ginzburg, 1960b, 1961a
	Salmo irideus	About 20		Devillers et al., 1954
				Kusa, 1956
	Salvelinus fontinalis			Kusa, 1956
	Coregonus laveretus ludoga			Lapitskii, 1949
	Plecoglossus altivelis			Kanoh, 1952b
		5–30		S. Ito, 1954

TABLE 1 (Continued)

Phylum, class, order	Species	Diameter (μ)	Chemical nature of contents	Author
	Hypomesus olidus (H. japonicus)			T. Yamamoto, 1954
			Polysaccharides	K. Yamamoto, 1955b
		16–40	Acid mucopolysaccharides	K. Yamamoto, 1955c
Cypriniformes	Leuciscus (Tribolodon) hakuensis			Kanoh, 1952a
	Rutilus (Leuciscus) rutilus		Acid mucopolysaccharides, proteins	Arndt, 1956, 1960a
	Abramis brama			Kryzhanovskii, 1953
			Acid mucopolysaccharides, proteins	Arndt, 1960a
	Abramis ballerus			Yurovitskii, 1956
	Vimba vimba		Neutral mucopolysaccharides, proteins	Sakun, 1960, 1961b
	Tinca tinca (T. vulgaris)		Acid mucopolysaccharides, proteins	Arndt, 1960a
	Barbus ticto		Neutral polysaccharides, proteins, glycogen	Chopra, 1958b
	Gobio gobio (G. fluviatilis)		Mucoproteins	Konopacka, 1935
	Acheilognathus lanceolata	4–15		Suzuki, 1958
	Acheilognathus tabira			
	Rhodeus ocellatus			
	Carassius auratus			Tchou-Su and Chen-Chao-hsi, 1936
			Mucopolysaccharides	K. Yamamoto and Yamazaki, 1961
	Cyprinus carpio		Proteins, polysaccharides	Konopacka, 1935
			Acid mucopolysaccharides, proteins	Arndt, 1956, 1960a
	Megalobrama terminalis			Tchou-Su et al., 1960
	Zacco platypus	5–35		S. Ito, 1954
	Brachydanio rerio	To 31	Polysaccharides	Malone and Hisaoka, 1963
	Lefua echigonia			Osanai, 1916
	Misgurnus anguillicaudatus		Acid mucopolysaccharides	Osanai, 1959
		5–10		Gamo et al., 1960
Cyprinodontiformes	Fundulus heteroclitus			Kagan, 1935; Marza et al., 1937
	Oryzias latipes	10–40		T. Yamamoto, 1939, 1944a
			Polysaccharides	Aketa, 1954
			Acid mucopolysaccharides	T. S. Yamamoto, 1955
Mugiliformes	Atherina boyeri		Proteins, polysaccharides	Konopacka, 1937
	Atherina hepsetus		Proteins, polysaccharides	Konopacka, 1937
	Melanotaenia maccullochi	To 30–40		Sterba, 1957

Gadiformes	Gadus morhua			Sars, 1876; Ryder, 1884
	Theragra halcogramma	5–10	Polysaccharides	Kanoh, 1954
Perciformes	Lucioperca (Stizostedion) vitreum			Reighard, 1893
	Perca fluviatilis	About 20	Polysaccharides, mainly hyaluronic acid	Mas, 1952, 1953
		About 20	Mucoproteins	Thomopoulos, 1953a
	Acerina cernua			Kryzhanovskii, 1953
	Smaris alcedo		Proteins, polysaccharides	Konopacka, 1937
	Ctenolabrus adspersus	1–6		Agassiz and Whitman, 1885a, b, 1889
	Crenilabrus pavo		Proteins, polysaccharides	Konopacka, 1937
	Tautoga onitis			Agassiz and Whitman, 1885a
	Ammodites tobianus	13	Mucoproteins	Thomopoulos, 1954
	Betta pugnax			Spek, 1933
	Ophiocephalus punctatus		Polysaccharides, proteins	Chopra, 1958a
	Channa maruleus		Acid mucopolysaccharides	Guraya, 1965
	Gobius paganellus		Proteins, polysaccharides	Konopacka, 1937
	Cottus bubalis euphrasen			Thomopoulos and Bauchot, 1957
Pleuronectiformes	Liopsetta obscura		Neutral mucopolysaccharides	K. Yamamoto, 1956b, c, f
	Limanda Schrenki			K. Yamamoto, 1951a
Gasterosteiformes	Gasterosteus aculeatus		Mucoproteins	Thomopoulos, 1953b
	Pungitius pungitius	4–25	Acid mucopolysaccharides	Kusa, 1953, 1956
	Pungitius tymensis	4–25	Acid mucopolysaccharides	Kusa, 1956
Amphibia				
Salientia	Xenopus laevis		Mucopolysaccharides	Voss and Wartenberg, 1954–1955; Wartenberg, 1956
			Neutral mucopolysaccharides	Wartenberg, 1959
	Bufo bufo (B. vulgaris) formosus		Acid polysaccharides	Osanai, 1960a
	Bufo stomaticus		Acid mucopolysaccharides	Guraya, 1965
	Rana temporaria (R. fusca)		Acid mucopolysaccharides	Wartenberg, 1956, 1959
		1–2	Acid mucopolysaccharides	Katagiri, 1959, 1960
		0.5–1.5		Wartenberg and Schmidt, 1961
	Rana esculenta		Slightly acid mucopolysaccharides	Wartenberg, 1959
	Rana pipiens			Kemp, 1956a, 1956b
			Acid mucopolysaccharides, proteins	Rosenbaum, 1958
		2–3		Kemp and Istock, 1964

TABLE 1 (Continued)

Phylum, class, order	Species	Diameter (μ)	Chemical nature of contents	Author
			Neutral and acid mucopolysaccharides, few proteins	Kemp and Istock, 1967
	Rana nigromaculata	About 1		Motomura, 1952
Mammalia				
Glires	*Cavia porcellus*			Szollosi, 1962
		About 0.5		Adams and Hertig, 1964
	Rattus rattus			Szollosi, 1962
	Mus musculus			Szollosi, 1962
	Cricetus (Mesocricetus) auratus	0.1—0.5		Austin, 1956a
			Polysaccharides	Yanagimachi and Chang, 1961
	Peromyscus maniculatus			Marston and Chang, 1966
	Myocastor coypus			Szollosi, 1962
Lagomorpha	*Oryctolagus cuniculus*			Szollosi, 1962
		0.08—0.2		Hadek, 1963b
Artiodactyla	*Sus scrofa*			Szollosi, 1962
Primates	*Macacus rhesus*	To 2.5		Hope, 1965

and resistence to osmium treatment was also noted in studying Golgi material in oocytes of many teleost species (see p. 8), so that these do not serve as basis for considering the argentophil bodies to be of different nature. In oocytes of plaice and herring small vacuoles which blacken on silver treatment emerge among the argentophil bodies at the end of the slow development phase; the vacuoles increase in size, and their contents show a distinct polysaccharide reaction. The participation of Golgi complexes in the formation of these vacuoles was later proved by an ultramicroscopic study of growing oocytes of goldfish (Yamamoto and Onozato, 1965). In vivo study of fish oocytes indicates that these polysaccharide-rich vacuoles stain with neutral red and may be identified with Parat's "vacuome" (Gerbil'skii, 1939; K. Yamamoto, 1955a, b; 1956c; Chopra, 1958a, b).

The polysaccharide-containing vacuoles always appear before yolk granules are formed, and in some species before the appearance of the first oil droplets but after in others. For example, in Gobius paganellus, Smaris alcedo, Crenilabrus pavo, Atherina boyeri, A. hepsetus (Konopacka, 1937), Fundulus heteroclitus (Marza et al., 1937), pike perch (Trusov, 1947, 1949), Pacific herring (K. Yamamoto, 1958b), snakehead (Chopra, 1958a), masu (K. Yamamoto et al., 1959), and Oryzias latipes (K. Yamamoto and Yoshioka, 1964) the vacuoles appear first, whereas in carp, gudgeon (Konopacka, 1935), Ludoga whitefish (Lapitskii, 1949), and many salmons (Persoy, 1966) the oil droplets appear first.

In some species — perch (Mas, 1953), Oryzias latipes (Aketa, 1954), and Lefua echigonia (Osanai, 1956) — the vacuoles initially are located immediatly next to the germinal vesicle; in carp, gudgeon (Konopacka, 1935), Crenilabrus pavo (Konopacka, 1937), Pacific herring (Yamamoto, 1955a, 1958b) they appear in various portions of the cytoplasm and then move to the periphery. In many fishes — Gobius paganellus, Smaris alcedo, Atherina boyeri, A. hepsetus (Konopacka, 1937), Fundulus heteroclitus (Guthrie, 1925; Marza et al., 1937), roach, bream, carp, tench (Arndt, 1956, 1960a), silver smelt (K. Yamamoto, 1956e), plaice (K. Yamamoto, 1956a, c, f), Volga shad and black-backed shad (Ivanov and Dodzina, 1957), Sakhalin stickleback (T. S. Yamamoto, 1963), Oryzias latipes (K. Yamamoto and Yoshioka, 1964), Channa maruleus (Guraya, 1965), pink salmon and sockeye (Persov, 1966), Sevan trout (Negonovskaya, 1966), the vacuoles occupy a peripheral position from the very beginning (Figure 3, a). In the oocytes of Melanotaenia maccullochi (Sterba, 1957) the vacuoles appear in the subcortical layer of the cytoplasm initially where the animal pole will develop, and then are gradually dispersed in the vegetal direction, as occurs in the spur-toed frog (see p. 30).

The number and size of the vacuoles increase in subsequent stages of oocyte development. New rows of vacuoles are added to earlier ones from the periphery toward the nucleus, until the vacuoles usually occupy most of the cytoplasm (Figure 3, b, e). The relative number of vacuoles varies in different species. For example, vacuoles are dispersed throughout the cytoplasm in gudgeon, carp, Smaris alcedo, and Crenilabrus pavo at the beginning of rapid growth phase, whereas in others (Gobius paganellus and two species of Atherina) there are never more than 2 or 3 rows of vacuoles which are located in the peripheral cytoplasm (Konopacka, 1935, 1937). At this time or somewhat earlier, yolk granules

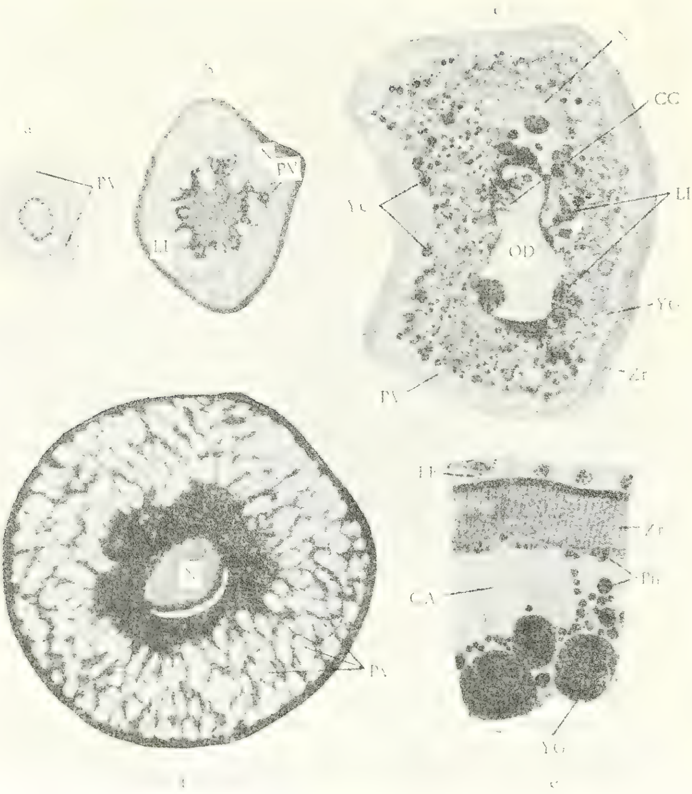

FIGURE 3. Accumulation of polysaccharide-containing vacuoles (precursors of cortical alveoli) in the oocytes of *Smaris alcedo* and *Rutilus rutilus*:

a — oocyte of *S. alcedo* on appearance of vacuoles; b — oocyte with cytoplasm packed with vacuoles, lipid inclusions appear; c — oocyte with eccentric nucleus and cortical alveoli visible at periphery; d — section of oocyte margin; e — oocyte of *R. rutilus* at stage of accumulation of polysaccharide-containing vacuoles (a—d, after Konopacka, 1937; e, after Arndt, 1960a). YG — yolk granules; OD — oil droplet; CA — cortical alveoli; LI — lipid inclusions; PV — polysaccharide-containing vacuoles; Ph — phosphatides; CC — cholesterol crystals; FE — follicular epithelium; N — nucleus; zr — zona radiata.

begin forming, first usually among peripheral vacuoles, then in the vacuole-free perinuclear cytoplasm. The vacuoles are gradually pressed toward the oocyte surface as the yolk granules increase in size and number; by the end of vitellogenesis the vacuoles are confined to the cortical layer (Figure 3, c, d). From this time onward, they are called cortical alveoli.

Since the vacuole layer narrows progressively as the oocyte grows, it would seem that they decrease in number. There are descriptions of the breakdown and dissolution of part of the vacuoles in studies of vitellogenesis (Konapacka, 1935, 1937) and even of gradual disappearance of all the vacuoles (Arndt, 1956). The latter is obviously erroneous; even Arndt, in following the fate of vacuoles, writes that most are preserved in the mature egg (Arndt, 1960a). Moreover, measurements of the thickness of the vacuolar layer (Osanai, 1956) indicate that not even part is destroyed. Osanai measured (with drafting equipment) the thickness of the vacuolar layer in oocytes of Lefua echigonia and calculated its volume. As the growing oocyte enlarges, the vacuolar layer initially grows rapidly; then, as yolk granules accumulate, it becomes progressively narrower (Figure 4,a). However, the space occupied by the vacuoles, after a rapid initial rise, remains unchanged or slightly increased (Figure 4,b). Thus, the vacuolar layer becomes thinner due to redistribution of the vacuoles along with the increased oocyte surface.

Investigators who observed the emergence and accumulation of vacuoles in teleost oocytes gave them various names: vesicles of unsaturated yolk (Guthrie, 1925), gouttes claires (Konopacka, 1935, 1937), yolk vesicles (K. Yamamoto, 1955—1958), Rindenvakuolen (Arndt, 1956), vacuolar yolk (Chopra, 1958a, b), intravesicular yolk bodies (Malone and Hisaoka, 1963), blue yolk (i.e., yolk stained with aniline blue) (Kazanskii, 1947), carbohydrate yolk (Guraya, 1965), vacuolelike bodies (Osanai, 1956), and, finally, simply vacuoles (Meien, 1927, 1939, 1940; Hibbard et Parat, 1928; Spek, 1933; Gerbil'skii, 1939; Trusov, 1947, 1949; Lapitskii, 1949; M. F. Ivanov, 1951; Anisimova, 1952; Protasov, 1952b; Ivanov and Dodzina, 1957; Chepurnova, 1958). Some of the authors consider them special inclusions playing some role in vitellogenesis or in the formation of the cortical layer, while others view them as direct precursors of yolk granules formed by condensation of the vacuolar contents. Only recently has the relation between the vacuoles and the cortical alveoli finally been proven by several investigators (Aketa, 1954; K. Yamamoto, 1955—1958; T. S. Yamamoto, 1955; Osanai, 1956; Sakun, 1960, 1961b; Arndt, 1960a), who traced the transformation of the vacuoles to cortical alveoli up into the fertilized egg where the contents are discharged under the membrane.

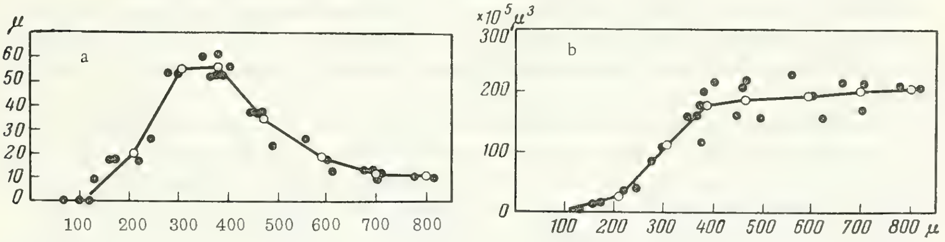

FIGURE 4. Thickness and volume changes in layer of vacuoles (precursors of cortical alveoli) in oogenesis of Lefua echigonia (after Osanai, 1956)

Abscissae: diameter of oocytes; ordinates: thickness of vacuolar layer (a) and volume (b).

Until the polysaccharide-containing vacuoles were properly understood, their formation was often considered as the initial yolk accumulation phase or was included in this phase (Meien, 1939, 1940; Trusov, 1947, 1949; M.F. Ivanov, 1951; Protasov, 1952a, b; Ivanov and Dodzina, 1957). This terminology is now obviously unacceptable. In many fish species, an initial rapid growth phase of the oocyte can be distinguished; this is the previtellogenesis phase during which the vacuoles — precursors of the cortical alveoli — appear and accumulate. In some species (e.g., in salmon, Ludoga whitefish, carp and gudgeon) no such phase exists since rapid growth begins with initial accumulation of fat before vacuoles appear.

Structure and chemical composition of cortical bodies in fish eggs. In the light microscope, the cortical bodies of sturgeons appear rounded or oval and not quite regular (see Plate XIV, b); the internal structure cannot be observed with usual cytological methods.

Study of the granules of Acipenser güldenstädti and A. stellatus with the electron microscope (Ginzburg, unpublished) indicates that these bodies vary both in shape and size (see Plate I, a, b; XI, a). Large granules can have a diameter of 3—4μ, while small granules measure 0.2—0.5μ and are invisible in a light microscope. The cortical granules consist of two fibrillar components; there are numerous inclusions of highly dense material in the lighter matrix. The granules are bounded by a membrane.

The cortical granules (Detlaf, 1957, 1961b, 1962) take several basic stains (methylene blue, toluidine blue, pyronine) and some acid stains (aniline blue and malachite green). These provide cytochemical tests for acid mucopolysaccharides: γ-metachromy on staining with toluidine blue, and also a clear Schiff reaction, which cannot be removed by treatment of microscopic sections with diastase. Since the granule contents are inactive to hyaluronidase, it may be assumed that the carbohydrate component is an acid mucopolysaccharide of the heparin type (see Pearse, 1960). Since the granules show a positive Schiff reaction not only with mixtures fixing polysaccharides but also with Sanfelice fluid, Bovin fluid, Helly's fluid and formalin and after heating of the sections in water, it may be concluded that their polysaccharides are present in the form of mucoproteins.

The cortical alveoli of teleosts are round or oval vesicles which deform on contact with one another. In an early work the cortical alveoli of Fundulus were described as disk-shaped platelets (Kagan, 1935) due to inaccurate observations; the cortical alveoli in Fundulus, as in other teleosts, are specific vacuolelike formations (Kao et al., 1951; Kao and Chambers, 1954). In many species the alveolar contents in fixed eggs are intensely colored with basic dyes (toluidine blue, thionine, neutral red, safranine) and show affinities to no acid dyes except aniline blue. When Azanov's method is used for studying vitellogenesis, according to Mallory, the cortical alveoli and precursors are selectively stained bright blue with aniline blue.

The alveolar contents extracted from Oryzias latipes eggs immediately after their formation are homogeneous and shapeless (Nakano, 1956a). The precursors of the cortical alveoli in Fundulus oocytes (Guthrie, 1928) appear to consist of an unstained substrate containing one or several rounded inclusions which intensely stain with neutral red, methylene blue and other vital stains. The cortical alveoli of cyprinids — roach, bream, etc. (Arndt, 1956, 1960a) — clearly show a complex structure upon fixation and even without treatment in certain alveoli. Within the alveolus (varying in diameter from 5.4 to 21.2μ) there are from 1 to 12 vacuoles (diameter 3.0—6.8μ) whose number depends upon its size. There are even smaller strongly refractive secondary vacuoles (diameter 1.0—2.5μ) inside the vacuoles. Histochemical tests show that the composition of the components of the alveoli varies.

The structure of the cortical alveoli is peculiar to each species. In the goldfish, the alveoli are filled with a compact mass containing numerous scattered vacuoles; in carp the alveoli contain a large number of small inclusions. In hybrids of carp and goldfish, the cortical layer of the oocyte has a mosaic structure: some alveoli appear like the paternal species, others like the maternal species (Takayama et al., 1961).

The contents of the alveoli invariably show a positive histochemical reaction to polysaccharides. In most species, the carbohydrate component, consisting of acid mucopolysaccharides, gives metachromatic shading with toluidine blue, thionine and polychrome methylene blue (chum salmon, sockeye, silver smelt, roach, bream, tench, carp, char, Oryzias latipes, etc., cf. Table 1). In Pacific herring, vimba, and plaice, neutral mucopolysaccharides are found in the alveoli. In certain fishes of the first group (chum salmon, smelt, char), the vacuolar precursors of the cortical alveoli contain neutral mucopolysaccharides in the early stages of rapid oogenesis, while acid mucopolysaccharides appear only at later stages. In Oryzias latipes, the vacuoles contain acid mucopolysaccharides from the very beginning.

There are data for perch that a considerable part of the polysaccharides in the cortical alveoli consists of hyaluronic acid (Mas, 1952, 1953). The acid mucopolysaccharides in chum salmon and sockeye are different, since lengthy treatment of microscopic sections with hyaluronidase does not substantially reduce staining (Kusa, 1954, 1956). The carbohydrate component of cyprinid alveoli cannot be related to sulfated acid polysaccharides (Arndt, 1960a); on the one hand, the alveolar contents stain with alcian blue and give a distinct metachromatic tinge to toluidine blue, polychromatic methylene blue and cresol red, characteristic for such polysaccharides; on the other hand, the metachromatism disappears after treatment with alcohol and is not found in acid medium. It has been suggested that the cortical alveoli of cyprinids contain both neutral and acid mucopolysaccharides, the latter in small quantities and with free carboxyl groups which are blocked on fixation and subsequent histological treatment.

The mucopolysaccharides of the cortical alveoli are apparently present in the form of mucoproteins. The mucopolysaccharides probably predominate over proteins, since histochemical reactions to proteins are negative (K. Yamamoto, 1956c) or weak when present (Kusa, 1954; Chopra, 1958a, b; Sakun, 1960). However, in some cyprinid species — roach, bream, tench, and carp — quite distinct reactions were obtained to the tryptophan, tyrosine (Millonov's reaction, reaction of tetrazo combination), and the α-amino monomers (ninhydrin method).

Acid hydrolysis of cortical alveoli of Oryzias latipes showed that the polysaccharide component predominantly contains galactose, along with mannose and a methylpentose-type sugar (Ohtsuka, 1958).

Cortical alveoli of Pacific herring contain disulfide-rich substances, as demonstrated by the ferricyanide method of Chevremon and Frederick (T.S. Yamamoto, 1957). Peroxidase is found in whitefish alveoli (Zotin, 1961a).

Cortical alveoli have considerable density: when eggs of Oryzias latipes, ninespine stickleback, and Pacific herring are centrifuged at low speeds, the alveoli move centrifugally (T. Yamamoto, 1944b; Kusa, 1953).

The alveoli are enveloped by a semipermeable membrane (Kusa, 1957b). This is clearly discernible in ultramicrographs of the cortical layer (Kusa, 1966) and in the phase microscope with isolated alveoli (Nakano, 1956a). This membrane was visible even in the ordinary light microscope when alveolar contents were liberated by piercing of the wall by a needle or osmotic rupture in hypotonic medium; in addition, a thin membrane reminiscent of erythrocyte ghosts has been observed (Kusa, 1958).

The alveolar membrane contains lipids — probably phospholipids as in the case of other cytoplasmic membranes (T. Yamamoto, 1951, 1956, 1962; Kusa, 1957b). This is supported by the fact that walls of isolated alveoli in Oryzias latipes and ninespine stickleback eggs are dissolved by such lipid solvents as chloroform, ethyl ether, benzene, toluene, and isoamyl alcohol, but resist neutral acetone; lipase (steapsase) dissolves membranes of isolated alveoli of Oryzias latipes, as do bee and snake venoms containing lecithinases. Ethylene glycol and basic dyes, soluble in lipids, penetrate into isolated alveoli of the stickleback egg.

The membrane lipids of the cortical bodies are not detected cytochemically either by osmium treatment, by Ciaccio's method, or by staining with Sudan red (Gerbil'skii, 1939; Kusa, 1953, 1956; K. Yamamoto, 1955a, b, 1956c; Detlaf, 1957, 1962). The absence of a positive reaction in studies with the light microscope may be due to the extreme thinness of the membrane. However, there are data that the wall cortical alveoli in Oryzias latipes eggs show a lipid reaction by Ciaccio's method (Aketa, 1954), but this was not confirmed (K. Yamamoto, 1955a).

Cortical inclusions in the eggs of various animals. Cortical bodies resembling cortical granules and alveoli of fishes have been found in the eggs of many animals. Such inclusions were described for representatives of six phyla: flatworms, roundworms, segmented worms, mollusks, echinoderms and chordates (see Table 1).

Flatworms. In a study of fertilization in 11 turbellarian species, the cortical granules stain intensely with eosin (Kato, 1940), appear in the cytoplasm after dissolution of the germinal vesicle, migrate to the periphery and, by the time of union with the spermatozoon, (at metaphase I), are distributed in 1 or 2 rows over the entire egg surface. After penetration of the spermatozoon, the granules fuse into a single layer which absorbs water and changes into a thin egg membrane.

Roundworms. In nematode eggs, there are large vacuoles whose contents separate from the cytoplasm on fertilization. This process has been followed in live free-living nematodes of the genus Rhabditis (Bütschli, 1873; Pal'chikova-Ostroumova, 1926) and Diplogaster longicauda (Ziegler, 1895). In nematodes the sperm-egg union occurs before the egg matures (at the first-order oocyte stage with undissolved germinal vesicle). By the time the first polar body is formed, the vacuolar contents are discharged from under the membrane.

Initial changes in the fertilized egg were studied in detail in Parascaris equorum (Van Beneden, 1883; Fauré-Fremiet, 1913; Makarov, 1953a, b, 1958; Fauré-Fremiet et al., 1954). There are two types of inclusions in this egg, which are distinguishable on fertilization, called hyaline spheres and light-refracting bodies by Van Beneden.

The hyaline spheres (sphères hyalines) correspond to the vacuoles described in free-living nematodes; they appear as light-colored vesicles up to 10—15μ in diameter. These inclusions are homogeneous in fresh eggs at 37—38°; at reduced temperatures and on fixation the contents are vacuolized. The hyaline spheres are situated not in the cortical layer of the unfertilized egg, but in the deeper layers of the cytoplasm. Only after the egg is activated do they appear at the surface; therefore, they can be considered as cortical bodies only conditionally.

When the egg reaches the lower section of the oviduct, the fertilizing spermatozoon penetrates. Immediately after this, a chitinous membrane begins to form on the surface of the maturing egg. The main component of the membrane — glucosamine — is apparently synthesized from the abundant glycogen in the egg cytoplasm. Simultaneously, but independently (Fauré-Fremiet et al., 1954), the hyaline spheres move to the periphery of the cytoplasm and are discharged from the egg. Afterwards, waxy, light-refracting bodies are excreted from the egg, forming a waxy inner membrane on the zygote. The hyaline spheres lie in the perivitelline space between the waxy envelope and the egg surface.

Concepts on the chemical nature of the hyaline spheres have undergone considerable changes. In early works (see Table 1) these inclusions were considered to consist mainly of inorganic matter (calcium phosphate) and only traces of protein, and were therefore represented as a quite unique case of formation of "mineral reserves" during oogenesis (Panijel, 1951). Later protein was indicated as the main component of these inclusions (Fauré-Fremiet et al., 1954). Hyaline spheres also contain inorganic matter loosely associated with protein, but the chemical composition has not yet been determined. The spheres do not contain nucleic acids or lipids. Polysaccharides have not been determined in hyaline spheres; the Hotchkiss (Fauré-Fremiet et al., 1954) and Shabadash-Hotchkiss reactions (Makarov, 1958) were negative. The contents of these inclusions were labile, dissolved in distilled water, weak alcohols, various acids and bases and certain salt concentrations; they are poorly preserved by most kinds of fixatives.

Some properties of the contents (high lability, negative reaction to polysaccharides) and behavior (migration to the egg surface after sperm penetration) of the ascarid hyaline spheres are similar to those of the hydrophylic colloidal globules in sturgeon eggs (Detlaf, 1961b, 1962, see pp. 57, 237). When they first appear, they are more like the cortical bodies of fishes and sea urchins which are also formed in young oocytes long before maturation of the egg (Fauré-Fremiet et al., 1954), inasmuch as the hydrophilic colloidal globules in sturgeon oocytes are connected mainly with the nucleoplasm entering the cytoplasm after dissolution of the germinal vesicle membrane. Migration of the hyaline spheres to the oocyte periphery occurs after the sperm penetrates during the maturation phase of oogenesis when migration of cortical bodies occurs in a number of other animals (for example, turbellarians, sea urchins). The absence of polysaccharides in hyaline spheres (which would distinguish them from cortical bodies of all animals studied) can hardly be considered conclusive from a few histochemical determinations, since the material in the hyaline spheres was very labile and weakly metachromatic when stained with methylene blue, Nile blue and cresyl brilliant dyes.

The ascarid hyaline spheres must be considered highly specialized organelles, and further research is required to discuss their similarity to special inclusions in other animals, whose contents are discharged from the egg during fertilization.

Roundworms. In the cortical layer of unfertilized Nereis limbata, N. dumerilii, and N. diversicolor eggs, there are numerous alveoli whose contents are discharged on fertilization (Lillie, 1911; Spek, 1930; Novikoff, 1939a; Pasteels, 1966; Fallon and Austin, 1967). The cortical layer in these eggs is much thicker than that of other animals studied; the alveoli are arranged in 5 or 6 rows and contain material with characteristic fibrillar ultrastructure.

The contents of the cortical alveoli of N. limbata are apparently rich in acid mucopolysaccharides, since the cortical layer in the unfertilized egg gives an intense metachromasy with toluidine blue not removable with alcohol (Kelly, 1954). Chemical analysis shows that more than 75% of the gelatinous substance discharged from the egg are sugars containing uronic acid (Ferry, see Costello, 1949).

Cortical bodies in the eggs of other polychaetes have also been described as containing acid mucopolysaccharides and proteins (see Table 1).

Mollusks. There are granules in the cortical layer and directly beneath it in mature eggs of bivalve mollusks (see Table 1). These correspond to cortical granules of other animals in their location. Electron microscopy indicates that these are round or oval bodies covered with a double membrane and filled with fine granular material. Cytochemical tests indicate the presence of acid and neutral mucopolysaccharides. Centrifugation of unfertilized mussel eggs at 12,000 g for 20 min, causing stratification of the egg, does not shift the cortical granules.

The granules of bivalve mollusks have special features; in unfertilized eggs they tend to burst and their contents come into direct contact with the surrounding cytoplasm or even disperse into it. After fertilization the cortical layer of the egg in most species studied does not substantially change and the cortical granules are retained in it.

According to available publications the cortical granules in bivalve mollusk eggs resemble similar structures in other animals, both in location and chemical composition, but are distinguished by their behavior during fertilization. This point will be considered later when we examine the cortical reaction and the mechanism blocking polyspermy.

Echinoderms. The cortical granules of sea urchins have been thoroughly studied. They were first discovered by centrifugation of the eggs of Arbacia punctulata (E.N. Harvey, 1911); the granules stain with neutral red and were not displaced by centrifugal force. Even the first description indicated that the granules disappear on fertilization. Harvey believed that the granules form a substance which is discharged from the egg and hardens as a fertilization membrane. Later, cortical granules were found in intact eggs of other sea urchin species (Runnström, 1928; Hendee, 1931; Lindahl, 1932). They were shown to lie in the outer hyaline layer of the cytoplasm close to the egg surface, refract light strongly, and to be oval in shape (long axis oriented radially).

In most sea urchin species the cortical granules are difficult to discern in intact live eggs, being masked by pigments and yolk inclusions below the cortical layer. On centrifugation these inclusions move centrifugally, while cortical granules do not change position and remain clearly visible. Unlike cortical alveoli of teleosts, the cortical granules in sea urchin eggs are not displaced at all on centrifugation, not even by a strong centrifugal force (up to 10,000 g) which fragments the egg (Moser, 1939; E. B. Harvey, 1946). This resistance of cortical granules to centrifugation is assumed to be due to the viscosity of the surface cytoplasm of the mature egg, appearing in microdissection.

However, ultramicroscopic study of intact and centrifuged sea urchin eggs revealed no signs of a jellied surface cytoplasmic zone; according to one suggestion, the cortical granules remain at the egg surface on centrifugation, while the membrane bounding each granule is fused with the outer plasmatic membrane (Wolpert and Mercer, 1961, 1962).

When immature sea urchin oocytes are centrifuged, the cortical granules are concentrated centrifugally and thus appear to be the heaviest granular inclusions of the oocyte (Monné and Hårde, 1951). In Strongylocentrotus purpuratus the specific weight of the cortical granules is about 1.168 (Allen, 1957).

Cortical granules were described for 31 sea urchin species (see Table 1); they are the same stable component of the egg, as the cortical granules and alveoli are in acipenserids and teleosts. The granules of sea urchins vary from 0.5 to 2.0μ in size. Their distribution density also varies: in Strongylocentrotus purpuratus there are 42–52 granules per $100\mu^2$ of egg surface, in Clypeaster japonicus about 60, and in Strongylocentrotus pulcherrimus about 120 (Motomura, 1941; Endo, 1952).

Studies of oogenesis in sea urchins with the light microscope established that precursors of the cortical granules in young oocytes are formed near the nucleus, are then distributed throughout the cytoplasm, and move to the cortical layer only on maturation of the oocyte (Motomura, 1936, 1941; Monné and Hårde, 1951). Electron microscope research (McCulloch, 1952; Lansing et al., 1952; Afzelius, 1956a; Motomura, 1960; Y. Takashima, 1960) indicates that these precursors usually have a strictly regular organization — they contain concentric or spirally twisted lamellae (Figure 5, a). On egg maturation, the structure of the granules changes: at the periphery of the granule appear hemispherical or lenticular inclusions ("extralamellar bodies"), and the regular arrangement of the lamellae is disrupted. A microscopic section of the cortical granule of a mature egg (Figure 5, b). shows the remains of spiral lamellae in the center and peripheral inclusions embedded in the matrix, which in electron micrographs appears empty (Figure 5, b).

As in teleosts, the structure of cortical bodies in sea urchins shows distinct species specificity (Runnström, 1966; Lönning, 1967a). The cortical granules of various species differ in the relative density of peripheral inclusions and their dimensions, in the thickness and arrangement of lamellae in the central part and the ultrastructure of these components. In some species, e.g., Arbacia lixula, Temnopleurus toreumaticus, Echinocyamus pusillus, no traces of lamellar structure are found in mature egg granules. The similarities or differences in structure of cortical granules corresponds in many cases to the degree of taxonomic kinship of the species.

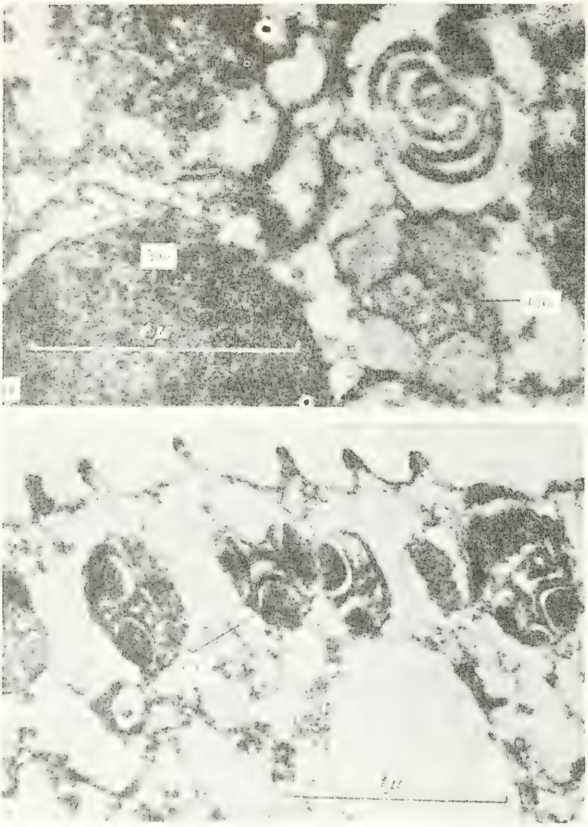

FIGURE 5. Cortical granules of Echinus esculentus:

a — two cortical granules in endoplasm of oocyte (upper, immature condition; lower, with peripheral inclusions); b — cortical layer of mature egg (according to Afzelius, 1956a). YG — yolk granules; CG — cortical granules.

Cortical granules are bounded by a double membrane, whose thickness varies in different species from 50 to 100 Å (Afzelius, 1956; Endo, 1961; Runnström, 1966). Destruction of the peripheral part of the granules is sometimes observed; in such cases the delimiting membrane is not closed, lamellar structures and peripheral inclusions are loosely arranged and their contours indistinct. Such changes are apparently associated with the progressive maturity of the eggs (Y. Takashima, 1960).

Cortical granules are intensely stained with Janus green (Motomura, 1941). Cytochemical research (Monné and Stautterback, 1950; Monné and Hårde, 1951) indicates that these bodies consist of proteins mostly, and acid mucopolysaccharides. The ratio between proteins and mucopolysaccharides apparently differs in different components of the granules; it may be assumed that proteins predominate in lamellar structures and peripheral inclusions and mucopolysaccharides, which are not preserved on fixation of the eggs with osmium tetroxide, in the matrix (see Runnström, 1966). The stainability of cortical granules with aniline blue and certain other acid dyes led us to suspect the presence of a small quantity of amino sugars (Monné and Hårde, 1951). They contain substances rich in sulfhydryl groups (Afzelius, 1956a).

Cortical granules are present not only in sea urchin eggs but also in the eggs of other echinoderms — sea lilies, startfishes and sea cucumbers (see Table 1). The granules in Asterias eggs, resembling those of sea urchins, are spherical with dense inclusions distributed at the periphery of their matrix.

Unlike those in sea urchins, the cortical granules in starfishes are easily displaced by centrifugation. If the egg is centrifuged at 6,000 g for 10 min and then fertilized, the membrane division and formation of the perivitelline space take place only in the centrifugal region (Costello, 1935), where the heaviest granular inclusions of the egg, the cortical granules, accumulate.

Chordates. Enteropneusta. The cortical layer of the egg of Saccoglossus kowalevskii contains 2–3 rows of small granules of unusual structure: in eggs fixed in Buen's fluid, half of the granules stains intensely and half weakly in hematoxylin (Colwin and Colwin, 1954a).

Tunicata. In ultramicroscopic studies of Ascidia nigra eggs (Ursprung and Schabtach, 1964), irregularly shaped flat vesicles filled with contents of low electron density were observed directly below the cell membrane. The nature of these vesicles and their fate after fertilization are unknown.

Acrania. Cortical alveoli of the amphioxus egg (Branchiostoma (Amphioxus) lanceolatus) were described at the end of the 19th century. Cortical alveoli in the ovarian eggs of Branchiostoma were first observed by Van der Stricht (1895), who took them for very small yolk granules. Having established that they decrease in size after fertilization and then become indiscernible, Van der Stricht thought that they were transported into the egg mass.

Sobotta (1897) demonstrated that these were cortical alveoli and not yolk granules (since they stain differently) and that after fertilization their contents are discharged from the egg. During the growth period of the oocytes, the cortical alveoli appear as light-colored vacuoles filled with fluid or semifluid matter. In the cortical layer of the mature egg there are 1–2 rows of transparent cortical alveoli which contain spherical bodies, separated by the delicate radial cytoplasmic strands.

In a study of oogenesis of Branchiostoma caribaeum, it was shown that precursors of the cortical alveoli appear at the periphery of growing oocytes before the beginning of vitellogenesis (Guraya, 1967). The cortical layer of large oocytes is stained with alcian blue and shows γ-metachromasia with azure B (Cowden, 1963) indicating the presence of acid mucopolysaccharides. No proteins were found in the alveoli; the membrane surrounding the alveoli gives a weak lipoprotein reaction (Guraya, 1967).

Vertebrates. Cortical bodies in fish eggs already have been described (pp. 10—24). Inclusions like cortical granules or alveoli have also been found in Cyclostomi, anuran amphibians and mammals.

In lamprey the rather large (7—13μ in diameter) cortical alveoli (see Table 1) are distributed over the entire surface except the animal pole and where the first polar body and second division spindle lie (Herfort, 1893; Kille, 1961) (Figure 1, a-c). The alveolar contents consist mainly of neutral mucopolysaccharides and give no distinct protein reaction (Kusa, 1957a).

The unfertilized egg in Anura contains much smaller surface cortical granules (see Table 1), which appear at the beginning of rapid development before pigment and yolk granules but after formation of the first fat inclusions. Initially a single row of cortical granules appears at the periphery; numbers then increase rapidly and are distributed in many rows in the cortical and subcortical layers. At these stages, the granules in Xenopus laevis are present only in the future animal hemisphere; in Rana temporaria and R. esculenta they are more numerous in this region than in the vegetal hemisphere. By the end of vitellogenesis the cortical granules are concentrated in a single row in the surface layer. In Xenopus laevis and Rana temporaria the granules in the animal region are masked by pigment granules and almost indiscernible but are clearly visible in the vegetal region. Due to lighter pigmentation in eggs of Rana esculenta the granules can be discerned over the entire periphery of the cytoplasm (Motomura, 1952; Voss and Wartenberg, 1954/1955; Wartenberg, 1956, 1959; Katagiri, 1959, 1960).

There are 17—18 cortical granules per $100\mu^2$ surface area in Rana temporaria (Katagiri, 1959) and about 20 in R. nigromaculata (erroneously given as 20 granules per $10\mu^2$ in Motomura, 1952). Thus, the density of these inclusions is much lower than in sea urchins.

Centrifugation of R. temporaria oocytes at various growth stages showed that cortical granules move centrifugally only before reaching a final position at the periphery of the ooplasm (Katagiri, 1960); the same was shown in mature eggs of R. pipiens (Rosenbaum, 1958).

Cortical granules of R. temporaria are round or oval, and flattened where they adjoin; ultramicrographs indicate that they have a uniform finely granular structure (Wartenberg and Schmidt, 1961). The cortical granules of R. pipiens contain particles with a diameter of about 100 Å enclosed in a less dense matrix (Kemp and Istock, 1967).

Histochemical tests of cortical granules of Anura indicated the presence of proteins and acid polysaccharides, in some species and neutral mucopolysaccharides in others (cf. Table 1). The acid mucopolysaccharides from the cortical granules of Rana pipiens are complexed with the protein (Rosenbaum, 1958). The cortical granules of R. pipiens and R. temporaria do not contain hyaluronic acid (Rosenbaum, 1958; Wartenberg, 1959; Wartenberg and Gusek, 1960b).

The thin membrane covering cortical granules contains lipids, as testified by the osmiophily in oocytes of R. esculenta (Wartenberg and Gusek, 1960a).

Ultramicroscopic studies showed that there are no cortical granules in oocytes of Urodela — Triturus alpestris and Ambystoma tigrinum (Wartenberg and Schmidt, 1961; Wartenberg, 1962).

No description of cortical granules in birds' eggs or alveoli in other animals were known until recently. Ultramicroscopic study of hen oocytes (Bellairs, 1965) revealed vacuoles in the cortical layer, which appear in the concluding growth phases. These vacuoles have dimensions similar (varying between 0.5 and 2.0μ) to those of amphibian cortical granules, but the similarity is merely superficial. The vacuoles in the hen oocyte are apparently derivatives of the cell membrane; their contents coagulate on fixation and form structureless lumps of extremely low density, indicating that they are filled with fluid of low organic content.

The ultrastructural investigations in the last decade of mammalian eggs have shown the presence of cortical granules in 9 species of 4 different orders (cf. Table 1). Human oocytes apparently contain cortical granules since such granules were found in derivatives of the first polar body at the stage of approach of the pronuclei (Zamboni et al., 1966). Cortical granules are probably present also in other representatives of this group. The maximum dimensions of cortical bodies in different mammalian species vary between 0.2 and 2.5μ.

In Cricetus auratus a large number of granules of uniform structure and with no inclusions (Austin, 1956a, 1961) are distributed relatively regularly over the entire yolk surface at a density of 50 to 100 granules per $100\mu^2$. In 7 mammalian species studied by Szollosi (1962), the cortical granules were structureless or consisted of closely packed homogeneous dense particles.

No cortical granules were found in oocytes of unripened follicles in rabbit; apparently they are formed only after stimulation by ovulation (Hadek, 1963b).

A comparison of data on cortical inclusions in the eggs of segmented worms, bivalve mollusks, echinoderms and chordates indicates that they occupy similar locations and have similar chemical composition in which polysaccharides predominate (acid in invertebrates, acid or neutral in vertebrates) with a certain amount of protein. These inclusions vary considerably in size (cf. Table 1): the cortical granules of mammals are 0.2—2.5μ in diameter, 0.5—3.0μ in amphibians and echinoderms, 3.2—4.0μ in Acipenseridae, whereas cortical alveoli in teleosts even reach 35—40μ.

Too little is known about cortical inclusions in flatworm and roundworm eggs to judge the degree of their relationship to similar formations in other animals (the chemical composition of hyaline spheres in the Parascaris equorum egg indicates their peculiarities).

The process in which contents of cortical bodies are discharged from the cytoplasm on fertilization plays an important role in protecting the egg from penetration by supernumerary spermatozoa (see Chapters 5 and 6).

Yolk. During oogenesis the fish oocyte accumulates a large number of deutoplasmatic inclusions, such as yolk granules ("protein yolk") and isolated lipid inclusions ("fatty yolk") in most fishes. The yolk granules of the "protein yolk" contain, in addition to proteins, a considerable quantity of lipids (as shown below, pp. 49, 58, 73).

All fishes have yolks of the telolecithal type; that is, yolk granules are distributed irregularly and concentrate in the vegetal pole of the egg, whereas there are fewer in the animal pole and a greater portion of the cytoplasm is yolk-free.

The most complex and involved questions of cytology pertain to the origin of yolk inclusions. Conventional microscopic methods have given extremely inconsistent results in various animals (see reviews of Wilson, 1925; MacBride and Hewer, 1931; Srivastava, 1965) particularly with respect to fishes. Yolk granules have been described as being formed with the participation of or from mitochondria (Narain, 1930; 1937; K. Yamamoto, 1955b, 1956d, 1958a; Chopra, 1960; Malone and Hisaoka, 1963), Golgi bodies (Wheeler, 1924), Parat's "vacuome" (Hibbard and Parat, 1927, 1928; Nath and Nangia, 1931; Konopacka, 1935; Nath, 1957), nucleoli material discharged into the cytoplasm (Singh and Boyle, 1938; T. Ito, 1938) and even from the cytoplasm (Chopra, 1958a). Many researchers believe that the lipid inclusions are formed with the participation of Golgi material (Narain, 1930, 1937; Nath and Nangia, 1931; Subramanian and Ayar, 1935; Nath, 1957; Chopra, 1960). However, data concerning vitellogenesis in the threespine stickleback and snakehead indicate formation of lipid inclusions independently of the Golgi complex (Singh and Boyle, 1938; Chopra, 1958a).

Assumptions based on the studies mentioned seem conclusive because yolk inclusions initially are so small as to be beyond the limits of resolution of the light microscope. Thus, conventional cytological methods are unsatisfactory for determining the origin of yolk inclusions. One of these suggestions is obviously incorrect, namely, the formation of yolk granules in the fish oocyte from elements of Parat's "vacuome"; the vacuoles appearing in the cytoplasm at the onset of rapid growth and vitally stained with neutral red are identical with the precursors of cortical alveoli, and yolk granules arise independently outside of these formations.

Electron microscopy undoubtedly enables deeper penetration into the process of vitellogenesis. Initial ultramicroscopic research has confirmed in fact that there are diverse paths of yolk granule formation which may differ not only in different species but even in the same animal.

In Poecilia (Lebistes) reticulata, formation of yolk granules has been shown to proceed in three different ways: from material concentrated in Golgi vesicles, in cisterns of the endoplasmic reticulum, and from exogenous material entering the oocyte by pinocytosis (Droller and Roth, 1966). In anuran amphibian, data conclusively testify to the mitochondrial origin of protein yolk and fatty yolk inclusions (Ward, 1959, 1962; Lanzavecchia, 1961; Balinsky and Davis, 1963); in addition, the formation of yolk granules in the cytoplasm (Kemp, 1956a) and the transformation of multivesicular bodies into yolk inclusions (Lanzavecchia, 1961) have been described. Data were obtained on the diverse origin of the yolk granules in invertebrate — with the participation of mitochondria, the Golgi complexes, endoplasmic reticulum and the cytoplasm and from exogenous material entering the oocyte by pinocytosis (see Beams, 1964; Aizenshtadt, 1965).

In order to elucidate the path of yolk granule formation in fishes, ultramicroscopic research on vitellogenesis in various objects is required.

The yolk inclusions formed in fish eggs show great differences in shape, size, structure and distribution. These features will be dealt with in the section devoted to egg structure in various groups of fishes.

1.1.3. Egg Membranes

Vertebrate egg membranes are divided into primary, secondary and tertiary, according to their origin. Primary membranes are derivatives of the oocyte itself; secondary membranes derive from follicular cells surrounding the oocyte in the ovary; tertiary membranes derive from secretions formed in the oviduct (see Brachet, 1935; Ivanov, 1937; Nelsen, 1953). All three types of membranes are found in fish eggs.

There is extensive literature dealing with the structure of fish egg membranes (early research has been reported by Hoffman, 1881; Waldeyer, 1906 and Retzius, 1912; see also Becher, 1928; Meien, 1939; Kryzhanovskii, 1949; M. F. Ivanov, 1956, 1958; Wickler, 1956, 1957; Sterba, 1957, 1958; Arndt, 1960c; Müller, 1962; Sterba and Müller, 1962; Müller and Sterba, 1963). Only certain basic questions will be mentioned below.

In most fish eggs, the zona radiata membrane, having radial striation, mainly protects the egg from external influences. This membrane is of a primary origin (see below). It is therefore incorrect to call it a chorion as some researchers do, since "chorion" is a long-established term for the secondary egg membrane in insects and its application to the fish zona radiata would only be misleading (see Wilson, 1925).

The distinctive structure of the zona radiata was first noted in eggs of char by Vogt (1842), who described numerous small rings on the intact egg surface and regular opaque dots on the isolated membrane. Vogt concluded that the membrane is pierced by thin tubules through which water enters the fertilized egg; however, evidence of the existence of these tubules, to which the zona radiata owes its radial striation, was obtained later.

In particular, F. V. Ovsyannikov (1885), who studied egg membrane structure in many teleosts, observed dye (aniline blue) penetrating into the zona radiata and silver nitrate solution depositing metallic silver in the tubules. These observations led him to conclude that "the role of the tubules must involve the supply of nutritive fluid to the yolk." Considering the hollow tubules in the zona radiata, Ovsyannikov suggested calling it the zona perforata.

Becher (1928) filled the zona radiata tubules with dye solution, air and paraffin and furnished conclusive evidence of their hollow structure.

Despite this evidence, certain researchers have continued until recently to maintain that there are no tubules in the zona radiata and that the striation is an optical illusion due to the material's columnar structure or, in modern terms, the radial arrangement of the macromolecules (Spek, 1933; Sterba, 1957, 1958; Siegel, 1957—1958). This opinion was finally disproven by ultramicroscopy which showed that the striation of the zona radiata of fish eggs, as in other vertebrates, consists of hollow tubules containing in ovarian eggs outgrowths of the oocyte and of the follicular cells through which nutritive substances enter the growing oocyte.

There were disagreements concerning the origin of the zona radiata. Most researchers consider this membrane to result from differentiation of the cortical layer of the oocyte, but some (Chaudry, 1956; Wickler, 1956) have suggested that it is secreted by the follicular epithelium. Evidence of secretory activity by the follicular epithelium in early stages was used to support the secondary origin of the zona radiata (Chaudry, 1956).

Such data, however, do not directly prove the direct participation of the follicular cells in formation of this membrane.

Ultramicroscopic research on egg membrane development has brought us much closer to an understanding of this process.

The development of the zona radiata was traced in detail in Cynolebias belotti (Cyprinidontidae) (Müller, 1962; Sterba and Müller, 1962; Müller and Sterba, 1963). At the time when vacuoles — precursors of cortical granules — appear, the oocyte surface produces numerous outgrowths (microvilli) at whose bases appears the first rudiment of the egg membrane in the form of a thin homogeneous structureless layer. This membrane is formed directly at the oocyte surface and at a distance from the follicular epithelium (Plate II, a). Simultaneously with the appearance and accumulation of yolk granules, a single layer of different structure is formed between the thin homogeneous layer (the first rudiment of egg membrane) and the cytoplasmic surface. This layer consists of regular clusters of tubular structural elements, each 200—600 Å in diameter (Plate II, b).

The authors following Spek (1933) and Arndt (1960c) unsuitably named this the "cortical layer" (Kortikalschicht), although its extracellular origin was obvious (at all stages it is separated from the cortical layer of the cytoplasm by the surface plasm of the oocyte). The inner structured layer of the membrane passes directly into the homogeneous outer layer. At the end of vitellogenesis, clusters of filiform elements become discernible, and both layers form a single membrane with almost homogeneous ultrastructure.

From its very initiation the egg membrane of C. belotti is pierced by tubules containing the oocytic microvilli, but these tubules are so thin (diameter about 0.07μ) that they are indiscernible under a light microscope. During vitellogenesis the tubules widen 3—4 times and the membrane's characteristic radial striation becomes visible under the light microscope. The follicular cells form outgrowths later than the oocyte, which permeate the tubules of the rapidly thickening zona radiata (Plate II, b), at an advanced stage of vitellogenesis when the tubules are completely occupied by the oocytic microvilli.

Thus the ultramicroscopic study on Cynolebias belotti indicates (as did light microscope data obtained with oocytes of Melanotaenia maccullochi (Sterba, 1957) and Rutilus (Leuciscus) rutilus (Arndt, 1960c)) that the zona radiata develops continually from material coming from the oocyte. Similar ultramicroscopic results were obtained in Fundulus (Kemp and Allen, 1956a), trout (Flügel, 1964) and representatives of the Syngnathidae — Hippocampus erectus and Syngnathus fuscus (Anderson, 1967).

The material forming the zona radiata most probably is not only excreted by the oocyte but synthesized by it. This is the picture observed at oogenesis in H. erectus and S. fuscus where a secretion accumulates in the Golgi complexes and glandular endoplasmic reticulum, secretion-containing vesicles move toward the plasma membrane and attach to the forming layer (Anderson, 1967).

The results of ultramicroscopic investigation show conclusively that the zona radiata of teleost eggs is a primary membrane.

The fully formed zona radiata consists of intercellular matter pierced by radial tubules within which the oocytic microvilli twist spirally and with peripheral follicular outgrowths. It is thus comparable to the zona radiata of amphibians and the zona pellucida of mammals. The suggestion that follicular outgrowths pass directly into the microvilli of the oocyte as cytoplasmic bridges (Kemp and Hibbard, 1957; Kemp, 1958) has not been confirmed in ultramicroscopic studies of fishes (Müller, 1962; Sterba and Müller, 1962; Müller and Sterba, 1963; Flügel, 1967) and other vertebrates (Kessel and Kemp, 1962). Apparently nutritive substances are excreted by the follicular outgrowths into the intercellular space from which they are taken up by the microvilli; such a mechanism has been determined for other types of animal cells.

Some early investigations on mature oocytes of various fishes mistakenly described a thin structureless "vitelline membrane" as being located between the zona radiata and the cytoplasm surface (Vogt, 1842; Lereboullet, 1854; Ransom, 1868, etc.). This most probably actually was the yolk-free cortical layer of the ooplasm. Even Hoffmann (1881) indicated that isolates of this layer, sometimes observed in preparations, were artifacts. It was later shown conclusively that the zona radiata directly adjoins the oocyte surface.

The adaptation through phylogenesis of the egg to conditions of development has led to considerable changes in the organization of the membranes in different fishes. Moreover, membranes are more affected by these adaptations than any other part of the egg. Membranes are simplest in teleosts, having pelagic eggs in which buoyancy is essential and external damage minimal; they generally consist of only one thin zona radiata. The eggs of lungfishes, chondrostean and holostean fishes, as well as teleostean bottom spawners, are constantly subjected to mechanical damage and usually have a well developed zona radiata and an additional outer membrane. This membrane consists either of a single homogenous or villous layer or else of isolated filamentous outgrowths. In some fishes the outgrowths arise throughout the zona radiata, in others they are confined to a limited portion of its surface. In the water, the outer membrane of the egg or the outgrowths swell and become sticky, serving as a perfect adaptation for attaching the eggs to plants and other substrates. In most cases these structures are of secondary origin, being secreted or directly formed from follicular cells (Eggert, 1929; Meien, 1939; M. F. Ivanov, 1956, 1958; Arndt, 1960c). In some fishes, however, there are indications of a primary origin of the villous membrane or of the isolated appendages of the zona radiata (Sterba, 1957; 1958; Müller, 1962).

Unlike in other fishes, the eggs of elasmobranchs have well developed tertiary membranes, in addition to a thin membrane formed in the ovary and apparently a modified zona radiata.

Micropyle. The egg membranes of fishes consist of dense material and are sperm-impermeable. Therefore, all fish eggs which are inseminated in the external milieu when membranes are fully formed contain micropylar openings through which the spermatozoa penetrate (Figure 6). In sturgeon eggs there are several micropyles (see Figure 15), while holostean and teleostean eggs have only one.

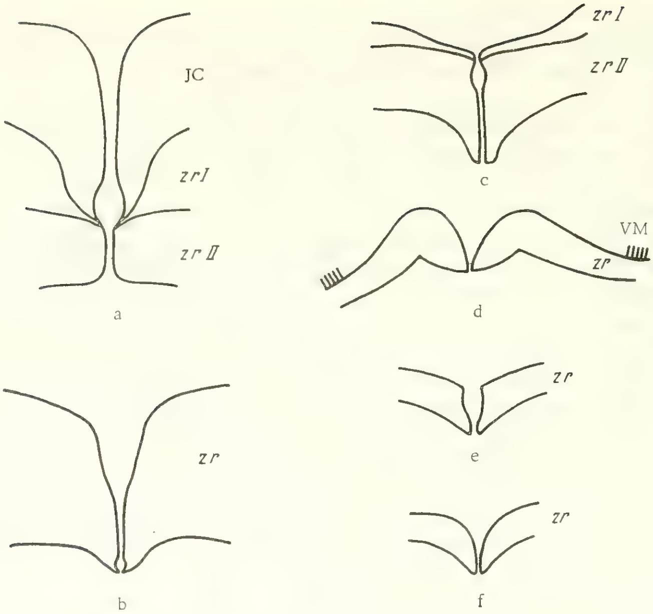

FIGURE 6. Section of egg membranes in region of micropyle:

a — *Acipenser güldenstädti colchicus*; b — lake trout; c — Atlantic herring; d — roach; e — tench; f — *Crenilabrus griseus* (a, b, —original; c—f — after Hoffman, 1881). VM — villous membrane; JC — jelly coat; zr — zona radiata; zrI — outer zona radiata; zrII — inner zona radiata.

Karl Baer first described the micropyle in 1835 in the egg of *Blicca bjoerkna*, but he was not aware of its significance since he failed to notice that the micropylar canal reaches the surface of the cytoplasm. Somewhat later, in 1850, Doyère observed the micropyle in the egg of *Syngnathus ophidium* and correctly assessed its role as an opening for sperm penetration without giving proofs for his view (see Ransom, 1868). Micropylar canals have been observed in the eggs of several other kinds of teleosts — trout, salmon, pike, *Chondrostoma* (Bruch, 1955a, b). Bruch described the organization of the micropyle and attempted unsuccessfully to follow microscopically how the spermatozoon penetrates the egg. The role of the micropyle in the fertilization process was proven experimentally in 1868 by Ransom who placed an unfertilized stickleback egg in a glass chamber and covered the micropyle by pressing it to a cover glass. Although spermatozoa had access to the rest of the egg surface, the egg remained unfertilized. If the micropyle was not covered, the egg became fertilized and cleavage was observed; the same occurred when the glass covering the micropyle was removed (Ransom, 1868).

Several features of the organization of egg membranes in various groups of fishes will be examined in section 1.2.

1.1.4. Composition of the Egg

Data on the chemical composition of fish eggs published up to 1930 have been summarized by Needham (1931, 1963) and later data, mainly of Soviet researchers (regrettably, without references), by Kleimenov (1962). Table 2 gives some information about the chemical composition of the eggs of various fishes. Most valuable for comparison are data obtained on the mature, ovulated eggs (stage V) or, at least, on oocytes at a definitive size (stage IV) since the chemical composition of oocyte changes substantially during gametogenesis (undoubtedly this is one of the main reasons for the discrepancies in analyses of the same material by different investigators). The table does not include data on immature oocytes, fertilized eggs and fixed eggs or where fish species were not mentioned. In cases where several determinations were given, averages were calculated.

Fish eggs contain a large amount of water, varying from 44 to 84% of the wet weight (see Table 2); in some species this percentage is even higher in fully mature eggs. The water content in fish eggs of various groups will be discussed later. The protein content of the egg displays considerable variations, from 12% of wet weight in redfish and 14% in Caspian salmon, burbot and surmullet, to 29—30% in trout, Rutilus rutilus, R. frisii kutum, Chalcalburnus chalcoides, Vimba vimba and catfish. There is even greater variation in the content of fatty substances, from 1—2% in Baltic herring, pike, crucian carp, cod, burbot, etc., to 22% in Scyliorhinus caniculus and 27% in Lepidosteus osseus. Proteins generally predominate over fats. Particularly rich in fatty substances are eggs of Scyliorhinus, sturgeons, Lepidosteus and certain salmons and smelts; in these fishes the ratio of protein to fat varies between 1 and 2. In most teleosts, the protein content is several or many times greater than that of fats and the ratio may reach 20:1 or even higher (pike, Baltic cod, pike perch).

Corresponding to the differing protein and fat contents, the energic properties of yolks in different fishes differ substantially (Faustov and Zotin, 1967), e.g., the caloric content of eggs of 12 fish species varies (in calories per gram dry weight) from 3,900 cal in Black Sea surmullet to 6,100 cal in salmon and 6,200 cal in Acipenser güldenstädti and A. stellatus. Among the kinds of eggs studied, demersal eggs had an average higher caloric value than pelagic eggs, and freshwater eggs are higher than those in seawater.

Proteins. The proteins of fish eggs have been poorly studied. Among them predominates ichthulin, which is soluble in salt solutions; a small amount of water-soluble albumin is also present (Table 3).

Comparative biochemical investigation of the main protein component of the eggs of Scyliorhinus stellaris, rainbow trout, green frog, hen and the invertebrate Sepia officinalis showed (Fujii, 1960) that it is consistently a lipophosphoproteid with similar properties in all vertebrates: insolubility in water or physiological salt solution, but soluble in more concentrated salt (1—3% NaCl) and alkali solutions (pH not less than 9); it has a sedimentation constant of 10 — 13 S, viscosity of 0.05 — 0.07, molecular weight of the order of $3.3 - 5.7 \times 10^5$; it contains 18 — 22% lipids. The main protein component of Sepia eggs has a much higher molecular weight (2.5×10^6) with substantially different properties than those of vertebrate egg lipoproteid.

TABLE 2. General chemical composition of fish eggs

Family	Species	Stage of maturity of ovary	Chemical composition (in %)				Protein/fat ratio	Author
			water	proteins	fats	ash		
Scyliorhinidae	Scyliorhinus caniculus (=Scyllium canicula)		53.15	23.58	22.34	0.93	1.06	Ford and Thorpe — after Needham, 1931, 1963
Acipenseridae	Huso huso		50.81	27.04	17.75	—	1.52	Drukker, 1932
			55.20	25.45	15.00	1.35	1.70	Mineev and Gerasimov, 1934
			58.17	23.29	14.62	—	1.59	Podsevalov, 1935
	Acipenser stellatus		49.94	27.84	18.19	4.03	1.53	Kiselevich, 1927
			49.61	27.05	16.47	—	1.64	Drukker, 1932
			53.0	26.8	15.5	1.35	1.73	Mineev and Gerasimov, 1934
			51.76	32.31	14.43	1.17	2.24	Podsevalov, 1935
	Acipenser nudiventris		55.79	24.91	12.79	—	1.95	Drukker, 1932
	Acipenser güldenstädti		56.52	24.93	14.14	4.41	1.76	Kiselevich, 1927
			56.10	26.60	12.95	1.30	2.05	Mineev and Gerasimov, 1934
	Acipenser güldenstädti persicus		55.10	23.91	13.20	—	1.80	Drukker, 1932
			58.16	25.91	14.20	1.59	1.82	Podsevalov, 1935
Lepisosteidae	Lepidosteus osseus		43.9	28.4	26.9	—	1.06	Nelson and Green, 1921
	Lepidosteus platystomus		53.9	26.2	18.3	—	1.43	
Clupeidae	Clupea harengus membras		76.39	20.17	1.96	1.09	10.29	Makarov, 1952
	Caspialosa kessleri pontica	IV	69.18	23.36	3.80	—	6.15	Vinogradskaya, 1954
	Caspialosa brashnikovi		72.24	22.13	2.44	1.71	9.18	Podsevalov, 1935
	Caspialosa caspica		73.55	22.83	2.30	1.67	9.73	Podsevalov, 1935
	Alosa sapidissima		71.25	23.44	3.78	1.53	6.20	Kiselevich, 1927
Salmonidae	Oncorhynchus tschawytscha	V	57.93	26.78	13.17	0.65	2.03	Green, 1921
			57.68	26.66	13.60	0.66	1.96	Kiselevich, 1927
	Salmo trutta caspicus		74.50	14.06	6.21	—	2.26	Podsevalov, 1935
	Salmo trutta m. fario	V	58.50	29.81	9.16	1.25	3.25	Fauré-Fremiet and Garrault, 1922a
	Salvelinus fontinalis		63.85	27.81	3.71	1.63	7.50	König and Grossfeld, 1913
	Stenodus leucichthys nelma		68.9	17.45	11.95	1.35	1.46	Kizevetter, 1949
	Coregonus autumnalis		62.85	16.3	16.2	1.4	1.01	Kizevetter, 1949
	Coregonus peled		62.5	17.6	15.1	1.6	1.17	Kizevetter, 1949
		IV–V	71.8	16.8	8.8	1.4	1.91	Medvedev, 1951
	Coregonus sardinella	IV–V	64.0	17.4	12.8	2.0	1.36	Medvedev, 1951
	Coregonus muksun		65.9	17.1	13.8	1.5	1.24	Kizevetter, 1949

		IV	60.1	21.9	12.8	1.2	1.71	Medvedev, 1951
	Coregonus lavaretus pidschian	IV	62.3	20.0	13.2	1.4	1.52	Medvedev, 1951
Osmeridae	Osmerus eperlanus dentex		62,3	23.3	11.6	1.7	2.01	Kizevetter, 1949
Eoscidae	Esox lucius		63.53	28.13	1.40	2.06	20.09	König and Grossfeld, 1913
			69.20	27.12	1.28	2.40	21.19	Kiselevich, 1927
			65.46	27.03	2.27	1.90	11.91	Podsevalov, 1935
			77,65	15.95	3.15	1.55	5.06	Kizevetter, 1949
Cyprinidae	Leuciscus idus		67.2	24.1	5.9	1.4	4.08	Kizevetter, 1949
		V	64.7	27.0	3.3	1.1	8.18	Medvedev, 1951
	Rutilus rutilus lacustris		65.6	25.25	4.85	1.7	5.21	Kizevetter, 1949
	Rutilus rutilus caspicus		71.15	24.28	2.57	2.00	9.45	Kiselevich, 1927
			62.36	29.69	1.26	—	12.07	Podsevalov, 1935
	Rutilus frisii kutum		59.49	30.27	2.06	—	14.69	Podsevalov, 1935
	Chalcalburnus chalcoides		59.88	29.20	5.43	1.6	5.38	Podsevalov, 1935
	Abramis brama		64.9	26.9	5.3	1.4	5.08	Veshchezerov, 1934
			65.03	27.14	3.34	1.62	8.13	Podsevalov, 1935
	Vimba vimba persa		58.99	30.24	3.31	1.21	9.14	Podsevalov, 1935
	Carassius carassius		69.5	23.4	1.8	2.0	13.00	Kizevetter, 1949
	Cyprinus carpio		66.15	27.70	2.48	1.40	11.17	König and Grossfeld, 1913
			66.3	25.7	6.2	2.0	4.15	Fauré-Fremiet and Garrault, 1922b
			73.96	21.72	2.12	2.20	10.25	Kiselevich, 1927
			67.7	25.0	3.7	—	6.76	Veshchezerov, 1934
			64.89	26.75	2.16	—	12.38	Podsevalov, 1935
Siluridae	Silurus glanis		61.0	30.0	3.6	—	8.33	Veshchezerov, 1934
			65.08	26.87	3.05	—	8.81	Podsevalov, 1935
Gadidae	Gadus morhua morhua		73.04	22.61	1.41	1.69	16.04	König and Grossfeld, 1913
			75.81	19.92	1.78	1.29	11.19	Turpaev, 1940
	Gadus morhua callarias	V	77.06	18.61	0.80	1.29	23.26	Kordyl, 1951
	Lota lota		81.4	13.8	1.9	1.6	7.26	Kizevetter, 1949
Percidae	Lucioperca lucioperca		71.01	25.56	1.18	2.25	21.66	Kiselevich, 1927
			69.07	17.05	9.27	1.34	1.84	Shul'man, 1934
			65.92	20.98	10.15	—	2.07	Podsevalov, 1935
	Perca fluviatilis		74.1	17.9	5.4	2.5	3.31	Kizevetter, 1949
Mullidae	Mullus barbatus ponticus	IV	77.40	14.12	6.46	—	2.19	Vinogradskaya, 1954
Gobiidae	Neogobius melanostomus	V	62.45	27.50	5.72	—	4.81	Vinogradskaya, 1954
Scorpaenidae	Scorpaena porcus	IV	84.28	12.00	1.06	—	11.32	Vinogradskaya, 1954
Atherinidae	Atherina hepsetus	V	72.67	18.69	3.23	—	5.79	Vinogradskaya, 1954

Salt concentrations required for dissolution of lipophosphoproteids from different fish egg vary: 2% in Scyliorhinus stellaris, 3% in the stellate sturgeon, 1% in rainbow trout (Feofilaktov and Karpov, 1937; Fujii, 1960).

Hardly anything is known about the protein microcomposition of fish eggs. Using paper electrophoresis for analyzing unfertilized eggs of Oryzias latipes, 5 bands were revealed: 1 band of phosphoprotein, 2 of lipophosphoproteins, and 2 more of proteins, each containing phosphate and inorganic pyrophosphatase (Ohi, 1963).

The proteins of the stellate sturgeon egg possess distinct acid properties — the isoelectric point of ichthulin lies at about 4 and that of albumin at about 5.1 (Feofilaktov and Karpov, 1937). In trout and perch eggs, the pH of the yolk was between 5.1 and 5.6 (Bourdin, 1926).

Fats. The fatty substances isolated from fish eggs are characterized by a high iodine number, indicating a high degree of unsaturation. Compared to fats of somatic tissues, the fatty substances of the egg contain much cholesterol and a very large amount of lecithin (Table 4). The fatty eggs of sturgeons mainly consist of neutral fats and about 20% cholesterol and lecithin. In teleost eggs, less rich in fats, lecithin and cholesterol constitute 50% or more of the total fats; it is interesting that among the 4 teleost species studied, separate fatty granules were found in only one (char) and here the total amount of fats was small.

Carbohydrates. Fish eggs contain an insignificant amount of carbohydrates. The content of reducing sugars (on conversion to glucose) is 0.34% in trout (Fauré-Fremiet and Garrault, 1922a) and 0.002 mg per egg or 0.5% in herring (Steudel and Osato, 1923b). Biochemical tests showed no glycogen in trout and carp eggs (Fauré-Fremiet and Garrault, 1922a, b; also see Needham, 1931, 1963), but with more recent micromethods the presence was established of about 0.04 mg of glycogen per egg in salmon eggs at cleavage (Daniel, 1947). Histochemical methods showed glycogen granules in the eggs of carp, gudgeon (Konopacka, 1935), salmon (Daniel, 1947), vimba (Sakun, 1960) and sturgeon (Detlaf, 1957).

Nucleic acids. In fish eggs, as in the eggs of many other animals, deoxyribonucleic acid (DNA) is found in amounts many times greater than in the diploid set of chromosomes; excess DNA is localized in the egg cytoplasm (see surveys of J. Brachet, 1960; Shmerling, 1965b; Grant, 1965; Kafiani and Timofeeva, 1967). According to the data of various investigators, the amount of DNA per egg is $0.1 - 1.5 \times 10^2$ diploid equivalents for sea urchins, $1.2 - 5.0 \times 10^3$ for fishes (Table 5), $0.2 \times 10^3 - 2.5 \times 10^4$ for amphibians, and 3.6×10^8 for hens. With respect to DNA content, fish eggs are very close to amphibian eggs, the quantity of DNA in the egg of different animals being proportional to the yolk reserves.

Cytoplasmic DNA is represented by a high and low molecular weight fraction. The heavy cytoplasmic DNA obtained from sturgeon oocytes (Shmerling, 1965a, b) had a molecular weight of $8 - 9 \times 10^5$ and was lighter than nuclear DNA from sperm heads of the same species (m.w. 5.2×10^6). The cytoplasmic DNA of the sturgeon in this preparation was bifilar, its molecular weight corresponded to the size of one or several genes and it could serve as a template for in vitro synthesis of DNA and RNA. Cytoplasmic DNA can apparently function as a template for in vivo synthesis of nucleic acids as well, as was corroborated particularly by investigations showing the initiation of synthesis of DNA and RNA in enucleated frog eggs (Mezger-Freed, 1963; Shmerling, 1967).

TABLE 3. Content of albumin and ichthulin in fish eggs (% of wet weight)

Fish	Albumin	Ichthulin	Author
Stellate sturgeon	2.5	17.0	Belinskaya et al., 1937
Sea sturgeon	3.32	13.87	König and Grossfeld, 1913, 1914
Herring	4.83	13.68	
Char	0.15	24.33	
Pike	2.38	17.29	
Cod	2.70	11.47	

TABLE 4. Fatty substances in fish eggs (after König and Grossfeld, 1913)

Species	Total fat content (% of wet weight)	Iodine number	Content in fat, %		
			cholesterol	diolein lecithin	cholesterol + lecithin
Sea sturgeon	15.36	107.6	4.35	12.92	17.27
Herring	4.19	123.1	6.94	43.61	50.55
Char	3.71	128.3	6.52	41.10	47.62
Carp	2.48	78.9	10.98	59.19	70.17
Cod	1.40	148.4	12.05	35.19	47.24

Investigations of recent years testify to the fact that heavy cytoplasmic DNA, or at least its greater part is localized in the mitochondria. It may be that low-molecular weight DNA — or deoxyribonucleotides — acts as a reserve which can be used by the developing egg (David, 1965, 1966).

Fish eggs also contain ribonucleic acid (RNA). The RNA phosphate content in the stellate sturgeon egg is on average 7.5μg per egg, or 17% of all the phosphorus in the egg; in the eggs of Huso huso it is about 20μg or about 22% (Kafiani et al., 1958). It is known that RNA contains 9.5% phosphorus; the stellate sturgeon egg must contain about 80μg RNA and the Huso huso egg about 210μg RNA.

Loach eggs contain considerably less RNA than the eggs of sturgeons. In the former, RNA phosphorus is about 6% of total phosphorus, and there is 2.4—2.6μg RNA per egg (Timofeeva and Kafiani, 1964).

Water. As already noted, fish eggs are considerably hydrated; the amount of water differs in different species of fishes. An extensive literature on this subject was compiled by Zotin (1961a, Chapter 5). A comparison of available data led Zotin to the conclusion that two groups of fish eggs can be distinguished according to water content: chondrostean and holostean fishes, belong to the first group, while the overwhelming majority of teleosts belong to the second. The first group is characterized by relatively lower water content: sturgeon eggs contain an average of 54.5% water, those of gar 48.9%. In teleost eggs (except for viviparous forms) the water content is always higher; the average amount for various ecological

TABLE 5. DNA content in the gametes of certain fishes

Species	Object of study	Method of determination	DNA content (in μg)		DNA in egg (in diploid equivalents)	Author
			in 1 egg	in 1 spermatozoon		
Huso huso	Egg at beginning of development	By phosphorus and deoxyribose in acid insoluble fraction	1.5*			Kafiani et al., 1958
Acipenser güldenstädti colchicus	Ovarian oocyte after removal of follicle by trypsin and dodecyl sulfate	By deoxyribose in acid insoluble fraction	1.8×10^{-1}			Shmerling, 1965a
	Unfertilized egg		1.9×10^{-1}			
	Fertilized egg		1.8×10^{-1}			
	Unfertilized egg				5,000	Shmerling, 1965b
Acipenser stellatus	Unfertilized egg and spermatozoon	By phosphorus and deoxyribose in acid insoluble fraction	9.0×10^{-1}*	2.3×10^{-6}		Kafiani et al., 1958
Misgurnus fossilis	Unfertilized egg and spermatozoon	Microbiological method	$2.5-3.5 \times 10^{-2}$	2.4×10^{-6}	5,000	Timofeeva and Kafiani, 1964
		By deoxyribose in acid insoluble fraction	2.0×10^{-2}		4,000	
		Microbiological method	5.8×10^{-3}	2.4×10^{-6}	1,200	Timofeeva, 1967
		By deoxyribose in acid-insoluble fraction	1.0×10^{-2}		2,000	

* The authors indicate that the method they used gives DNA values one order of magnitude too high.

groups varies between 63.7 and 92.2%. This difference is connected with the fact that developing sturgeon (holostean) eggs imbibe water from the surrounding medium, whereas teleost eggs (except viviparous fishes) contain the full amount of water required for development of the embryo.

The high variability of water content in teleost eggs is connected with the ecology of their development. Demersal eggs of marine fishes contain 60–75% water (average 70.0%), whereas pelagic eggs of marine fishes contain 85–97% (average 92.2%). The high water content in pelagic eggs is an adaptation for floating. Hydration of the yolk causes the density to be less (reaching 1.007–1.025 g/cm^3) than seawater (having a density of 1.026–1.028 at a salinity of 35‰ at 5–15°C); the density of demersal eggs varies between 1.056 and 1.081 g/cm^3. Strong hydration of pelagic eggs occurs at the end of oogenesis and is accompanied by an increase in oocyte volume by 3.6 times on the average, as indicated by Fulton (1898) for 24 marine species.

Demersal eggs of freshwater fishes contain 63.7–67.4% water, while pelagic eggs contain 72.3%. The buoyancy of such pelagic eggs is ensured by the fatty reserves and also by the formation of a large perivitelline space after fertilization or by pronounced swelling of the jelly coat; as a result the total water content of the egg together with membranes approaches 97–99% (Azov–Black Sea shad, Pelecus cultratus).

1.2. SPECIFIC STRUCTURE OF THE EGG IN DIFFERENT FISH GROUPS

1.2.1. Selachians

The specific features of egg organization in selachians (subclass Elasmobranchii, order Selachiformes) are closely connected with the conditions of reproduction and development of these fishes. In all selachians, insemination is internal. The female produces a small quantity of very large yolk-rich eggs; in some species these are spawned in water, in others they develop in a dilated section of the oviduct.

Egg shape. Mature ovarian and ovulated eggs have a regular spherical shape (Rückert, 1899). When fertilized, the eggs are surrounded by tertiary membranes and enclosed in a horny capsule, and their shape conforms to the configuration of the capsule. They usually become somewhat flattened and elongated along the long axis of the capsule, assuming the shape of a flattened ellipsoid. In cases where several eggs are enclosed in a single capsule, they are deformed due to mutual pressure (Figure 7, i).

Egg size. In different selachian species the egg size varies considerably, being determined greatly by conditions of embryonic development.

Eggs of selachians may reach colossal size: the mature eggs (when they become spherical) in Chlamydoselachus anguineus are 90–97 mm in diameter, those of Raja batis 70 mm, etc. (Table 6). Such a size is due to the accumulation of very large stores of yolk, an adaptation ensuring development of the embryo in the female over many months. A large yolk store is possessed both by oviparous and ovoviviparous species (which

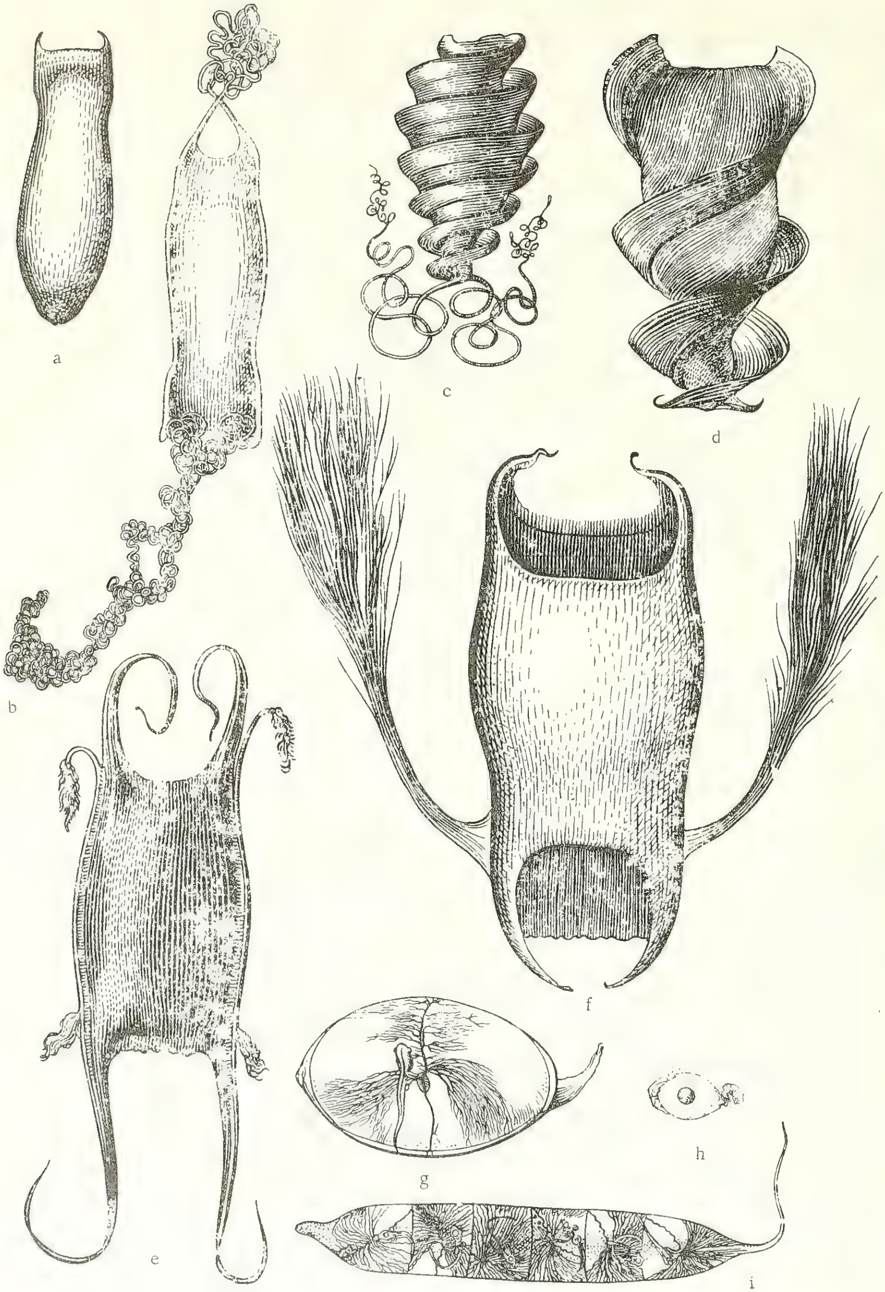

FIGURE 7. Egg capsules of selachians, oviparous (a—f) and viviparous (g—i):

a — Pristiurus melanostomus (after Smitt from Ehrenbaum, 1936); b — Scyliorhinus stellaris (after Wollebaek from Ehrenbaum, 1936); c — Heterodontus galeatus (after Waite from Smith, 1942); d — H. japonicus (after Smith, 1942); e — Raja erinacea (after Bigelow and Schroeder, 1953); f — R. batis (after Williamson from Ehrenbaum, 1936); g — Chlamydoselachus anguineus (after Nishikawa, 1898); h — Scoliodon sorrakowah (after Raghu Prasad, 1951); i — Squalus acanthias (after Graeffe from Ehrenbaum, 1936).

predominate among the selachians). In the latter group, the embryo develops intrauterinely, growing mainly on the yolk reserve in the egg without any direct connection to the maternal organism. In true viviparous selachians, special adaptations provide the embryo with nutritive substances. In many species — Torpedo ocellata, Trygon pastinaca, etc. (see Van den Broek, 1933; Budker, 1958 — the uterine walls form filiform outgrowths or villi, which secrete a fluid rich in organic substances; this "uterine milk" usually enters the cavity of the uterus and is taken up by the embryo there. In Pteroplatea micrura and Trygon pastinaca long villi (trophonemata) penetrate the spiracle into the esophagus of the embryo and secrete nutritive fluid directly into the digestive tract (Wood-Mason and Alcock, 1891; 1892; Disler, 1966). In some selachians of the suborder Carcharhinoidei (in the families Carcharhinidae and Sphyrnidae) a modified part of the embryonic yolk sac and the uterine wall are in close contact forming a yolk-sac placenta through which nutritive substances enter, gas exchange occurs and embryonic waste products are removed; such a placenta has been described for Mustelus canis (= M. laevis), Carcharias glaucus, C. melanopterus, Scoliodon palasorrah, S. sorrakowah, etc. (see Mahadevan, 1940; Harrison Matthews, 1955; Budker, 1958; Amoroso, 1960).

A distinctive adaptation for intrauterine feeding of the embryo arose in the family Isuridae (= Lamnidae). The store of nutritive matter contained in the yolk sac is rapidly used up by the embryo, so that the embryo feeds for a long period on undeveloped eggs passing from the ovary into the uterus (oophagy). The principal part of the stomach gradually increases in size, and the abdomen of the embryo swells up to colossal proportions: in Isurus oxyrhynchus (= Oxyrhina spallanzanii) 140 × 235 mm (Vaillant, 1889), in Lamna cornubica 123 × 211 mm (Lohberger, 1910). The ventral section of the embryo of Lamnidae, filled with yolk material, was mistakenly taken by early investigators to be the yolk sac; on this basis the impression was created that the eggs of Lamnidae are the largest eggs in the entire animal kingdom. Such a view, which may be encountered also in recent embryological literature, has been proven conclusively to be wrong (Swenander, 1907; Lohberger, 1910; Shann, 1923). The diameter of ovarian eggs of Lamnidae is unknown, but it is clear that their size does not exceed that of other selachians since an egg capsule containing two embryos of 15—16 mm long Lamna cornubica was 34 mm wide and 92 mm long (Vayssière, 1909).

When speaking of ovoviviparity and true viviparity, it should be remembered that a whole range of transitions exists both in selachians and teleosts, and that their demarcation is conditional. This justifies the point of view of certain investigators, such as Turner (1947), who call all species viviparous in which the fertilized egg remains in the maternal organism, even for a short time.

The development of adaptations insuring transfer of nutritive substances from the maternal organism is linked to progressive diminution of the yolk, reserves in the egg and, accordingly, the size of the egg. A clear example of such a correlation can be seen in Scoliodon sorrakowah, having the smallest known eggs of all selachians (see Table 6) in which a highly specialized placenta arises at early stages of embryonic development.

TABLE 6. Egg size in selachians

Species	Size (diameter or width × length), mm		Author
	eggs without tertiary membranes	egg capsule (without appendages)	
A. Oviparous			
Raja binoculata		88–122 × 190–265 (containing 1–5 eggs)	Hitz, 1964
Raja batis	70		Borcea, 1906
		77–145 × 143–254	Ehrenbaum, 1936
Heterodontus (Cestracion) japonicus	40–50		Dean, 1901
		Length 120–180	Smith, 1942
Raja laevis		62–72 × 124–132	Bigelow and Schroeder, 1953
Scyliorhinus stellaris (= Scyllium catulus)	25	40–43 × 110–125	Borcea, 1906
Raja clavata		49–69 × 63–90	Ehrenbaum, 1929, 1936
Chiloscyllium griseum	22*	35–40 × 75–80	Aiyar and Nalini, 1938
Raja radiata		35–51 × 44–68	Ehrenbaum, 1929
Scyliorhinus caniculus (= Scyllium canicula)	15		Borcea, 1906
		22–24 × 55–65	Ehrenbaum, 1929
Pristiurus melanostomus	15–17		Rückert, 1899
		~20 × 64	Ehrenbaum, 1929, 1936
Raja erinacea		34–45 × 55–63	Bigelow and Schroeder, 1953
Raja fyllae		24–28 × 40–44	Ehrenbaum, 1936
B. Viviparous			
Ginglymostoma cirratum		Length 133–180	Gudger, 1940
		130 × 180	Bigelow and Schroeder, 1948
Chlamydoselachus anguineus	62 × 100*	65–75 × 102–124 65 × 107	Nishikawa, 1898
	87–90 × 90–97 80–90 × 119–138		Dean (after Gudger, 1940)
Squatina angelus	60		Borcea, 1906
Rhinobatus granulatus Rhynchobatus djeddensis Pristis cuspidatus	About 50	120 × 250 (containing 3–5 eggs)	Raghu Prasad, 1951
Squalus acanthias (Acanthias vulgaris)	30		Borcea, 1906
Mustelus canis (M. laevis)	Up to 40		Berg et al., 1949
	17–20 × 30–38	60–80 × 350–480	TeWinkel, 1950
	20 × 30		Grodziński, 1958
Torpedo ocellata	20–25		Rückert, 1899
Scoliodon palasorrah	12 × 25		Mahadevan, 1940
Cetorhinus maximus	5		Harrison Matthews, 1950
Scoliodon sorrakowah	1	3 × 5	Raghu Prasad, 1951

* Diameter of yolk sac of embryo.

Egg structure. The yolk reserves filling selachian eggs consist of yolk granules and platelets enclosed in cytoplasmic stroma; in the few of the species studied, separate fatty inclusions are few or absent.

The germinal disc on the egg surface containing more active cytoplasm than the rest of the egg is distinguished by its color; it is red in Heterodontus japonicus and orange or bright yellow in other species. The rest of the egg is paler: greenish yellow in H. japonicus and Pristiurus melanostomus, pale yellow in Torpedo ocellata and pinkish in Chlamydoselachus anguineus (Nishikawa, 1898; Rückert, 1899; Dean, 1901; Gudger, 1940; Smith, 1942).

The germinal disc is shaped like a plano-convex lens with the flat surface facing outward toward the egg membrane. It occupies a small part of the egg surface (Figure 8); in mature eggs (up to or after fertilization) its diameter is 1.5—2.0 mm in T. ocellata (Rückert, 1899), 1—2 mm in P. melanostomus (Kashchenko, 1888b; Rückert, 1899), 1.2 mm in Scyliorhinus caniculus (Kashchenko, 1888b) and 1.3 mm in spawned eggs of C. angineus (Nishikawa, 1898).

The egg nucleus at the metaphase stage of the second maturation division is located at the surface of the germinal disc. The disc contains only small yolk granules, which in Torpedo do not exceed 2 μ in diameter (Rückert, 1899); it is surrounded by a zone of finely granular yolk, which gradually passes into coarsely granular yolk, filling the rest of the egg.

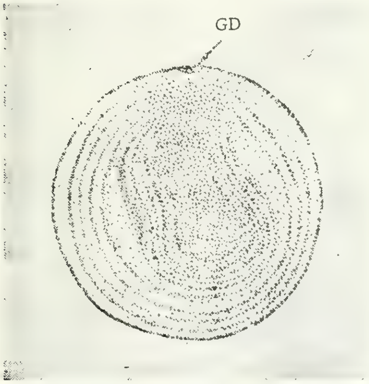

FIGURE 8. Egg structure in Torpedo marmorata, meridional section (after Rückert, 1899)

(GD — germinal disc).

The presence of large yolk reserves in the selachian egg indicates that cleavage is incomplete, and cleavage furrows divide the region of the germinal disc (Figure 9, a). A phenomenon uncommon among selachians has been described in Heterodontus japonicus in which cleavage is evident also on the yolk surface, similar to the cleavage pattern in gar eggs (Figure 9, b, c; compare Figures 9, c and 18, Lepidosteus). This was interpreted as indicating a tendency toward the ancestral type of cleavage and it was concluded that during phylogenesis selachian eggs attain large dimensions before losing their capacity for complete cleavage (Dean, 1901). Such an interpretation seems doubtful. First, it was not established how the cleavage described for Heterodontus japonicus resembles true cleavage; since the eggs were not studied in microscopic sections, it is not known whether the cleavage furrows penetrate deeply into the yolk. Second, there is no correlation between the position of the germinal disc and the center of the cleavage planes. Finally, cleavage furrows outside of the germinal disc were not observed in any selachian, even in representatives of the Heterodontidei (Haswell, 1916; Smith, 1942).

Yolk. As in birds' eggs, the yolk has a platelike structure, clearly discernible in Torpedo and Pristiurus melanostomus eggs, particularly when unfixed. Light-colored concentric layers are intersticed with narrow darker ones at the egg periphery; near the center the layering becomes less distinct, while at the very center there is a spot of darker yolk resembling the latebra in birds' eggs (see Figure 8). The difference between lighter and darker layers is determined not not by different types of yolk granules as in a hen's egg, but by differences in size and distribution of monotypic yolk platelets. The dark layer and central spot are formed by loosely arranged, larger yolk granules (Rückert, 1889).

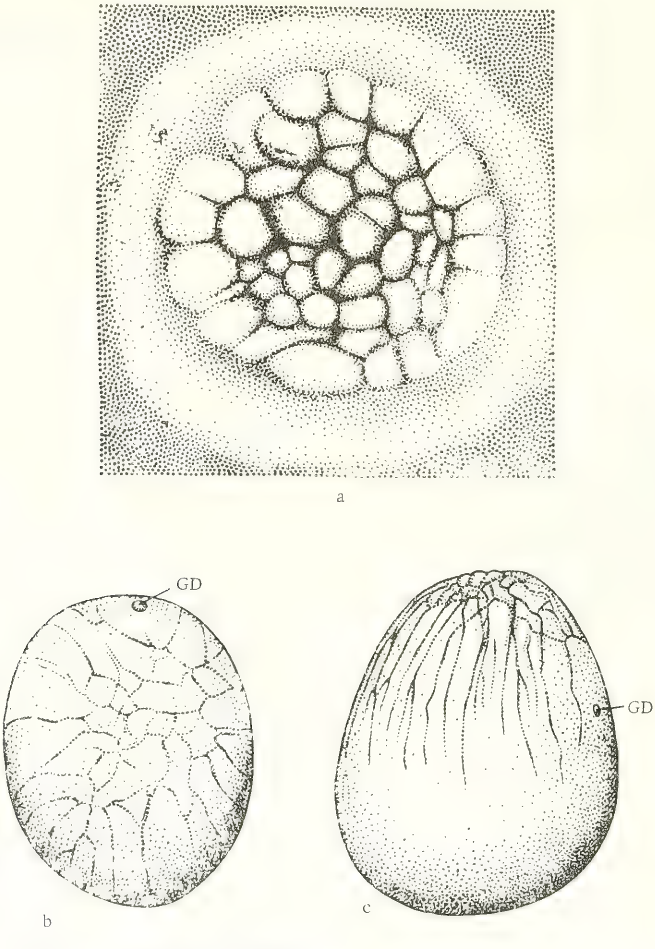

FIGURE 9. Egg cleavage in selachians:

a — blastodisc of Scyliorhinus caniculus egg at 64-cell stage; b, c — cleavage of Heterodontus japonicus egg; b — egg at moment of spawning, viewed from upper hemisphere; c — lateral view of egg several stages later (a — after Rückert, 1899; b, c — after drawings of B. Dean from Smith, 1942). GD — germinal disc.

The coarsely granular yolk inclusions are of complex structure. They consist of rectangular or elliptical shiny dense bodies surrounded by more fluid material which is weakly refractive. Rückert (1899) called these elements yolk platelets to distinguish them from yolk granules contained in the blastodisc. In different selachian species, the yolk platelets differ in shape and size. In Pristiurus melanostomus they are usually elliptical, more rarely rectangular or square; in Torpedo they are generally rectangular or trapezoidal; in Pristiurus most of them are 10—17μ (to 43) long, in Torpedo marmorata 13—27μ (to 35), in T. ocellata only 8—10μ (to 22).

The yolk platelet structure was described in detail for Raja batis (Fauré-Fremiet, 1933) and Mustelus canis, syn. M. laevis (Grodziński, 1958). In Raja batis the yolk platelets enclosed in fluid-containing vacuoles have a flat, square shape and, in a particular orientation, clearly showing double refraction; the anisotropy disappears after the yolk platelets are kept in alcohol, possibly due to dissolution of a yolk component. In weakly alkali solutions and sufficiently concentrated salt solutions (e.g., 10% NaCl) the yolk platelets swell in one direction; in this condition it can be seen that they consist of closely approximated rodlike elements. Anisotropic rods alternate with isotropic ones. After complete dissolution of the rods, the coat membrane appears to be not essentially different from inner components of the yolk platelet.

The yolk platelets are composed of material called "ichthin" and contain the protein ichthulin, phospholipids (lecithins), neutral fats and cholesterol, which constitute most of the fatty substances of the fat-rich selachian eggs, for instance, lipids constitute up to 17% of the dry weight of the yolk platelets in the egg of Raja batis and only a small part of these is concentrated in the few oil drops distributed in the yolk.

The structure of yolk platelets of the Mustelus canis egg resemble similar inclusions of Raja batis. A single yolk inclusion is globular or, more correctly, a vacuole about 30μ in diameter bounded by a semipermeable membrane. Inside, the vacuole is a rectangular platelet, 14×20μ and 2μ thick. The rest of the vacuole is fluid containing dissolved proteins and ultramicroscopic neutral fat particles. As in the Raja batis egg, the yolk platelet of Mustelus laevis consists of a membrane and a complex core. In hypertonic (0.5—2 M) NaCl solutions, the platelet swells and separates into layers of two different substances. One type of layer dissolves rapidly in salt solution, while the second type mixed among them, lemon yellow-colored and intensely light refracting, is more resistant. There are no isolated fatty inclusions in M. canis eggs.

Membranes. Only ovulated eggs of selachians are covered with a membrane, apparently a modified zona radiata.

Balfour (1878) showed in the case of Scyliorhinus caniculus and S. stellaris that this membrane first appears in the embryo as a delicate homogeneous layer on the oocyte surface. In the young female, a second layer with distinct radial striation is formed below the homogeneous layer. Both these layers are primary since the homogeneous layer appears before the follicular epithelium is formed and the striated layer is always separated from the epithelium by the outer homogeneous layer. During oocyte development, both layers thicken, but then are gradually reduced at the concluding stages of growth. In Raja both membrane layers appear, but the striated layer appears later and is less well developed than in Scyliorhinus. Balfour named the outer layer the vitelline membrane, and the inner layer the zona radiata. Later investigations on formation of teleost egg membranes, however, indicate that these two layers constitute together the zona radiata, but only the earlier outer layer contains the radial tubules (as in Cynolebias belotti, p. 34), that are so thin as to be indiscernible under a light microscope.

Young oocytes of Pristiurus and Torpedo, as well as Scyliorhinus, have a zona radiata of a considerable thickness with distinct radial striation (Rückert, 1892b, 1899). This membrane reaches maximal development in 1—2 mm-diameter oocytes, and then becomes gradually thinner: it is not more than 2μ thick in the mature egg of Torpedo, and it is even thinner in the egg of Pristiurus. At this stage the radial striation is preserved only in certain places, mainly above the blastodisc. The thin, weakly light-refracting layer below the zona radiata bordering on the yolk, described by Rückert (1899) as the vitelline membrane, is actually the undifferentiated cortical layer of the egg, since it never separated from the underlying cytoplasmic layers and is drawn deeply into the cleavage furrows.

The ovulated egg is moved along the body cavity by ciliary action of the peritoneal epithelium, enters the upper section of the oviduct, and descends in it to the shell gland. A protein substance is produced in the anterior part of this gland, gelatinous substance in the narrow intermediate region and capsule material in the posterior part (Metten, 1939; Raghu Prasad, 1945; Budker, 1958); thus, the egg is surrounded by tertiary membranes, the chalaza ("membrane chalasifère" — Wintrebert, 1927a, b), a semifluid protein substance and the solid capsule. The selachian egg capsule and gelatinous fluid around the egg correspond to the shell and albumen layer of birds' eggs.

The shell gland commences secretion before the ovulated egg reaches it, so that the caudal half (or even three-quarters) of the capsule is already formed by the time the egg, surrounded by protein substance, slips into it; the remaining anterior part of the capsule is then formed rapidly (Hobson, 1930; Metten, 1939).

The fresh capsule material is white and soft, and gradually becomes darker and assumes a horny consistency. Despite previous ideas, the capsule proteins differ substantially from typical keratins and may possibly be closer to collagens; the capsule hardens by transformation of its proteins (Brown, 1955; Krishnan, 1959).

In oviparous selachians the capsule reaches maximum development and has a complex structure (Fauré-Fremiet, 1938; Smith, 1942; Bigelow and Schroeder, 1953; see also Ehrenbaum, 1929, and Bertin, 1958a). For example, the capsule wall in Scyliorhinus caniculus, Raja batis and R. undulata consists of 4 concentric layers. The general shape of the capsule is usually rectangular, and at the corners conical processes are formed by the inner layer that closely adjoins the other layers which form a border along the edge and 4 cirrilike (Scyliorhinus) or antennalike (Raja) processes (see Figure 7, b, c, e, f). The length of these processes differs considerably in different species; sometimes they are small or only one of the two pairs develops. In Scyliorhinus the cirri are more than

1 m long when extended. The capsule in Port Jackson sharks (Heterodontidae) is distinguished from other selachian eggs by conical shape and two spirally twisted lamellar processes (Figure 7, c, d). In some species the capsule has numerous soft fibrous appendages (see, for example, Figure 7, f). The processes and appendages of the capsule serve to attach the eggs to a substrate.

The capsule color varies from amber yellow or greenish to bottle green, dark brown and black. The protein substance in the capsule wall has a special structure; individual layers consist of numerous plates or tubule systems alternating with longitudinal and transverse layers. Even if the capsule wall is relatively thin — 0.32 mm in Scyliorhinus caniculus and 0.75 mm in Raja batis (Fauré-Fremiet, 1938) — it is quite firm.

Most capsules of oviparous selachians contain one egg each, but a single capsule may sometimes contain several; for example, Raja binoculata capsules contain from 1 to 5 eggs, usually 3 or 4 (Hitz, 1964).

The transition to oviparity is accompanied by progressive reduction of the shell glands (Raghu Prasad, 1945). In some ovoviviparous species (e.g., in Rhinobatus granulatus and Rhynchobatus djeddensis), those shell sections secreting protein and capsule material are well developed, but mucus glands disappear. In true viviparous species, the reduction may also be confined to the mucus glands (Carcharinus melanopterus) or also include the protein glands (Scoliodon palasorrah and S. walbeehmi). The next stage is characterized by complete loss of mucus and protein glands, and a considerable reduction of the capsule-secreting zone (Scoliodon sorrakowah). Finally, the shell glands disappear completely in some viviparous species (e.g., in Narcine timlei, N. brunnea, Astrape dipterygia).

Along with reduction of the shell glands, the capsule wall becomes thinner and simpler. In ovoviviparous Rhinobatus granulatus and Rhynchobatus djeddensis the capsule wall consists of two thin and one more strongly developed layer; the capsule wall is thin, transparent (Figure 7, h) and consists of a single homogeneous compact layer (Raghu Prasad, 1951) in Scoliodon palasorrah, S. sorrakowah and three other truly viviparous sharks of the Arabian Sea. The egg capsule is absent in the species having complete reduction of the shell glands.

In some viviparous selachians (e.g., Chlamydoselachus anguineus, Ginglymostoma cirratum, Mustelus canis, Scoliodon sorrakowah) each egg has its own capsule (Figure 7, g, h), whereas in many species (from the genera Squalus, Squatina, Spinax, Rhinobatus, Pristis and Trygon) the capsules contain several, usually up to 6—8, eggs (Figure 7, i); capsules of Trygon pastinaca were found to contain 12 eggs (Disler, 1966). Reduced appendages often develop at the ends of the capsule in place of cirri or antennae (Nishikawa, 1898; Gudger, 1940; Raghu Prasad, 1951; Disler, 1966); such appendages are shown in Figure 7, g—i.

The capsule formation in selachians in which embryos develop intrauterine and the gradual reduction of the capsule in transition from ovoviviparity to true viviparity leave no doubt that this group initially reproduced by spawning of eggs protected by a firm capsule which developed outside the maternal organism.

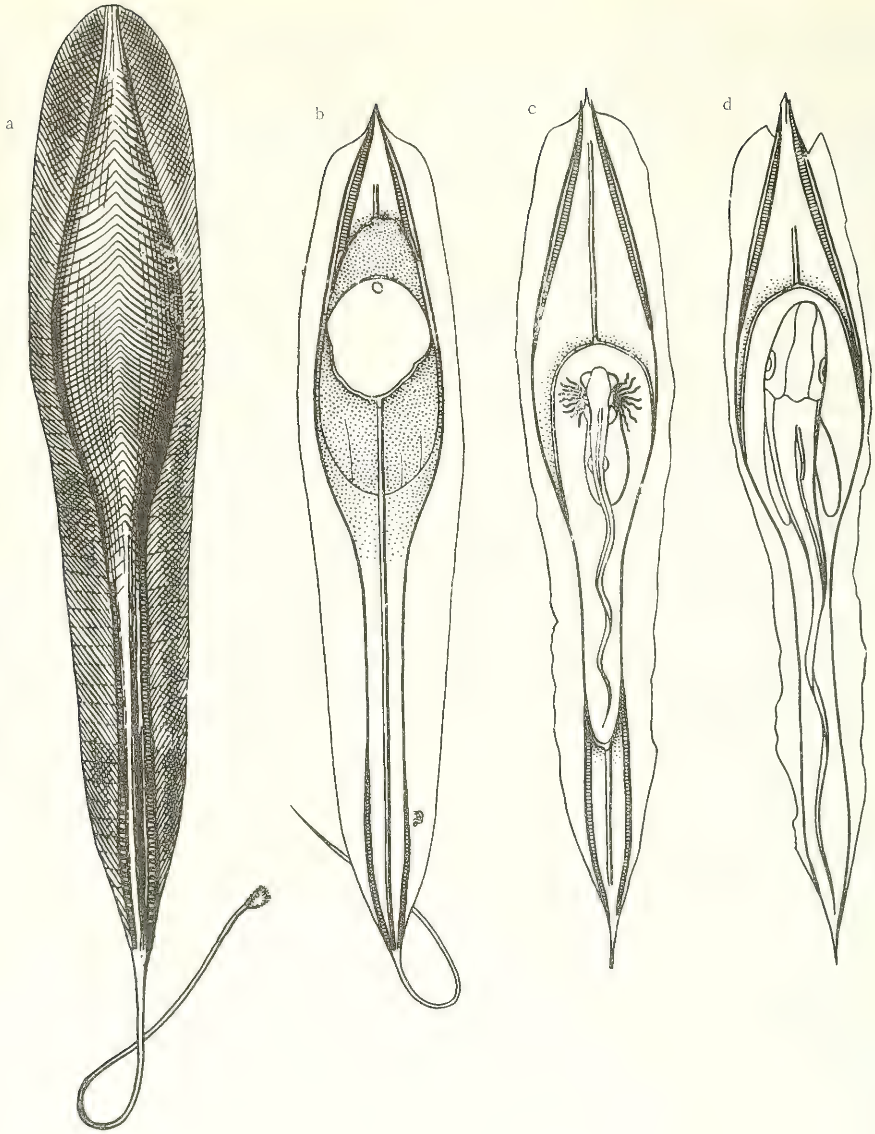

FIGURE 10. Egg capsule of Hydrolagus colliei:

a — external view; b—d — capsules dissected to show developing embryo at final blastula stage (b), with well developed outer gills (c), and shortly before hatching (d) (after Dean, 1903, 1906).

1.2.2. Chimaeras

Chimaeras (subclass Holocephali, order Chimaeriformes) are closely related to selachians in reproduction, conditions of egg development and egg structure. Insemination is internal. The eggs, which contain a large reserve of yolk, are covered by a firm capsule and are deposited in pairs in the water.

During oocyte development, the zona radiata becomes very thick and has a typical structure; but as in selachians, it is greatly reduced at the end of development; for instance, in Hydrolagus (Chimaera) colliei the thickness of this membrane decreases from 6 to 1.5μ (Stanley, 1963).

As in selachians, the capsule is multilayered but differs in its shape; the inner chamber is fusiform and often elongated in the posterior part (Figure 10). The outer capsule wall layers form a wide border, sometimes covered with filiform appendages. The egg capsule in Chimaera monstrosa reaches a length of 163–177 mm (Ehrenbaum, 1929) and in Callorhynchus antarcticus a length of 250 mm (Parker and Haswell, 1938).

Initially, the egg occupies only a small part of the capsule; the capsule in Hydrolagus colliei (Figure 10 b) is about 180 mm long, whereas overall dimensions of the egg are 29×19×13 mm (elliptical, and slightly flattened). As the embryo develops (apparently this takes about a year), it fills the whole space of the capsule (Figure 10, c, d), whose structure is adapted more to the needs of the developing embryo than to the egg (Dean, 1903, 1904, 1906).

1.2.3. Lungfishes

In the lungfishes (subclass Dipnoi, order Ceratodiformes) insemination is external. Many eggs, slightly smaller than those of most selachians (from 3 to 7 mm in diameter, see Table 7) are spawned. The spherical demersal eggs are deposited among vegetation (Neoceratodus) or in special nests (Lepidosiren, Protopterus).

TABLE 7. Sizes of lungfish eggs

Species	Egg diameter, mm		Author
	without jelly coat	with jelly coat	
Lepidosiren paradoxa	6.5–7.0	–	Kerr, 1900, 1909
Protopterus aethiopicus	4.5–5.0	–	Greenwood, 1958
Protopterus dolloi	4.0	–	Pasteels, 1962
Protopterus annectens	3.5–4.0	–	Budgett, 1901
Neoceratodus forsteri	3.0	6.5–7.0	Semon, 1903

Data on egg structure are very scant. The main mass of yolk inclusions is concentrated in the vegetal pole, which comprises the greater part of the egg and differs in color from the animal pole. In the Australian lungfish (Neoceratodus forsteri) the eggs are greenish gray and the animal pole is darker (Semon, 1903); in Lepidosiren paradoxa, on the other hand, the animal pole is lighter, almost white, and the vegetal pinkish orange like the color of yolk granules (Kerr, 1900). Lungfish eggs resemble those of chondrosteans in the yolk concentration, but are somewhat more primitive as indicated by their complete and irregular cleavage (Figure 18).

During oogenesis in Protopterus, a typical zona radiata first develops around the oocyte and then is gradually reduced (Beddard, 1886).

Lungfish eggs are apparently covered with tertiary membranes in the oviduct. An ovulated egg of Leposiren taken from the body cavity was surrounded only by a transparent 1-mm-thick membrane whereas spawned and fertilized eggs are covered by a thin, firm membrane, resembling a horny capsule, outside of which is a jelly coat resembling that of frogs' eggs (Kerr, 1900). Like in anurans, the jelly coat of lungfish eggs can be quite thick; in Ceratodus the egg itself is about 3 mm in diameter, the egg together with the jelly coat is 6.5–7.0 mm (Semon, 1903).

1.2.4. Chondrostean Fishes

In chondrostean fishes, acipenserids and spoonbill sturgeons (subclass Teleostomi, superorder Chondrostei), fertilization is external. The eggs are spawned in rapid rivers, so that at the spawning grounds the demersal eggs attach to underwater substrates (pebbles, stones, etc.) and develop attached (see Detlaf and Ginzburg, 1954; Purkett, 1961).

Egg shape and size. Acipenserid eggs are round or, more often, somewhat elongated, often ovate with a slightly compressed animal end (Figure 11). Their maximum diameter is from 1.9 mm in Acipenser ruthenus to 4.0 mm in Huso huso and H. dauricus (Table 8). Among normal-sized eggs there is sometimes a small percentage of much larger ones, probably polyploids (Detlaf and Ginzburg, 1954). This was observed in several egg batches of Acipenser stellatus and A. güldenstädti colchicus.

One spoonbill sturgeon, Polyodon spathula, has eggs of similar shape (Ballard and Needham, 1964) and size (Table 8).

General structure of the egg. Acipenserid eggs are characterized by distinct polarity in the irregular distribution of the cytoplasm and yolk and pigment inclusions. The upper animal pole represents less than one-third of the egg and is clearly distinguished by pigmentation from the lower vegetal pole (Figure 11). The animal pole contains much active cytoplasm, small yolk granules and lipid inclusions, whereas the vegetal pole contains coarsely granular yolk and large oil droplets (Figure 12, 14). Due to the presence of a fairly well developed cytoplasmic stroma between the yolk inclusions of both the animal and vegetal poles, egg cleavage is complete; moreover, the uneven distribution of these inclusions determines the irregularity of the cleavage (see Figure 18).

Egg color. The egg is usually brownish gray of varying hues and intensity from light yellowish gray to pure black. The eggs of Acipenser stellatus, Huso huso and A. ruthenus are lighter than those of A. güldenstädti. Particularly strongly pigmented eggs are found in

Acipenser sturio (Ryder, 1890) and A. güldenstädti colchicus. Albino specimens of sturgeon with completely white eggs are occasionally caught (Nedoshivin, 1928; author's own observations). The eggs of Polyodon spathula are dark gray-brown (Larimore, 1950; Ballard and Needham, 1964).

A light, sometimes completely white, polar spot is located at the center of the animal region of sturgeon eggs (Figure 11); it is surrounded by a ring of dark pigment. Another pigment ring is generally formed at the boundary with the vegetal pole. Between these, there is often one more intermediate ring. The width and intensity of pigmentation of the dark rings and lighter regions between them varies greatly even in a single batch of eggs. The eggs of Acipenser güldenstädti colchicus are sometimes so strongly pigmented that individual rings become indiscernible (Figure 11, b).

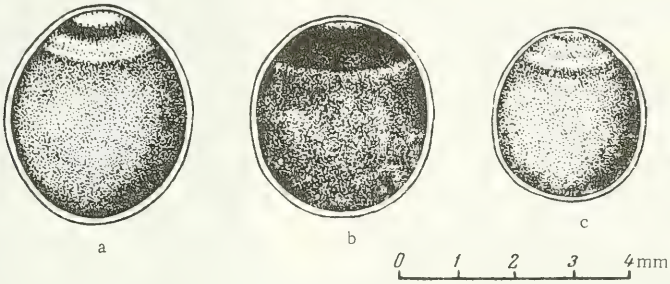

FIGURE 11. Eggs of Huso huso (a), Acipenser güldenstädti colchicus (b), and A. stellatus (c), lateral view (after Detlaf and Ginzburg, 1954)

When eggs become overripe, changes occur in the pigmentation pattern of the animal pole. The pigment begins contracting toward the pole, the size of the light polar spot diminishes and the pigment becomes wide and distinct. Such eggs are still fertile. With progressive ripening, the pigment accumulates in the center of the animal pole and becomes very light, almost white. These changes are associated with loss of fertility (Detlaf and Ginzburg, 1954). The pigment changes during overripening correspond to those observed after fertilization.

The vegetal pole has a distinct, rather intense color, usually (but not always) darker that that of the animal region.

The color of sturgeon eggs is determined by the presence of a large quantity of pigment granules in the peripheral cytoplasmic layer (see Plate I, a, c). These granules do not dissolve in acids or in fat solvents, so they may be assumed to consist of melanin (Grodziński, 1963).

Nucleus. The nucleus at metaphase of the second maturation division is located in the area of the light polar spot; the spindle is perpendicularly or slightly obliquely to the oocyte surface (Ginzburg, 1957a, 1959). Numerous small chromosomes are visible in the equatorial plane of the spindle, their paired arrangement is clearly discernible. The chromosomes stain intensely with nucleic dyes and show a distinct positive Feulgen reaction.

TABLE 8. Egg size in chondrostean and holostean fishes

Species	Diameter of egg with membranes, mm	Author
Chondrostei		
Huso huso	3.6 – 4.0	Detlaf and Ginzburg, 1954
Huso dauricus	3.6 – 4.0	Berg et al., 1949
Acipenser güldenstädti colchicus	3.2 – 3.8	Detlaf and Ginzburg, 1954
Acipenser güldenstädti	3.0 – 3.5	Berg et al., 1949
Acipenser stellatus	2.7 – 3.2	Detlaf and Ginzburg, 1954
Acipenser sturio	2.6 – 3.0	Berg et al., 1949
Acipenser baeri	2.5 – 2.7	Sokolov, 1965
Acipenser ruthenus	2.0 – 2.5	Zavadskii, 1926
	1.9 – 2.0	Berg et al., 1949
Polyodon spathula	2.0	Larimore, 1950
	2.7 – 4.0	Purkett, 1961
Holostei		
Lepidosteus osseus	3.5	Fülleborn, 1894
	3.3	Dean, 1895, 1896a
Amia calva	2.6 – 2.8	Dean, 1896a, b
	2.5 – 3.0	Whitman and Eycleshymer, 1897

The spindle lies beside the maturation depression, remaining at the place of division of the first polar body; the polar body itself lies above the depression and is usually flattened (Figure 13). The spindle is generally right next to the maturation depression or a very slight distance from it, although sometimes this distance is tens of microns (25–28μ in Huso huso). In rare cases, the spindle is located below the maturation depression, touching it at one end, as described for lamprey (Herfort, 1893, 1901, see Figure 1, b).

Surface layer of cytoplasm. In mature, unfertilized sturgeon eggs the layer of cytoplasm adjoining the egg membrane contains cortical granules arranged usually in a single row (Plate XIV, b). The layer containing pigment grains lies deeper. Both cortical and pigment layers are wider in the animal pole of the egg than in the vegetal.

The cortical granules are distributed over the entire surface, even close to the spindle of the second maturation division; they usually are visible in the cytoplasm at the base of the maturation depression, but lie farther apart here than in other sections of the cortical layer. Sturgeons differ in this respect from lampreys (Herfort, 1893, 1901; T. Yamamoto, 1944c) and most teleosts in which the cortical alveoli are not found near the meiotic spindle.

Study of such eggs (Ginzburg, unpublished) in the electron microscope showed a slitlike space about 0.5μ wide between the surface cytoplasm and egg membrane into which protrude numerous microvilli rising from the cortical layer; the surface cytoplasmic layer, about 10μ thick, is free from yolk inclusions (Plate I, a). Immediately below the membrane lie cortical granules; some (Plate I, b) are right next to this membrane, while others are located some distance away. The pigment granules lie deeper (Plate I, c).

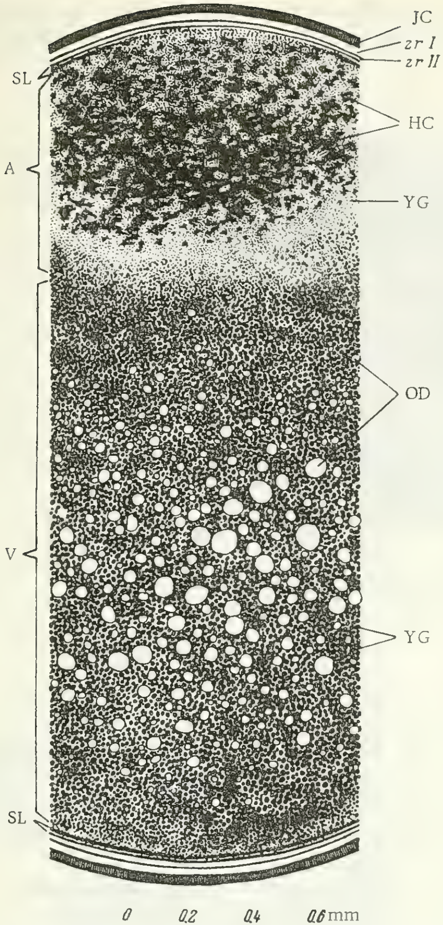

FIGURE 12. Structure of sturgeon egg (after Detlaf and Ginzburg, 1954):

A — animal pole; V — vegetal pole; HC — spaces containing hydrophilic colloid; YG — yolk granules; OD — oil droplets; zrI — outer layer of zona radiata; zrII — inner layer of zona radiata; SL — yolk-free cytoplasmic surface layer; JC — jelly coat.

as well as numerous elongated and rod-shaped mitochondria of typical structure (Plate I, d). Immediately beyond the limits of the section shown in Plate I, a, there are inclusions of protein yolk and fatty yolk. In the cytoplasm between these inclusions are numerous small vesicles and free ribosomes.

Hydrophilic colloid in spaces of animal pole. In mature unfertilized eggs, a large number or irregularly shaped lacunae joined together in places can be distinguished in the cytoplasm of the animal region (Figure 12). These spaces were first described in the egg of Acipenser ruthenus (Zalenskii, 1878), and later for other sturgeons — Acipenser güldenstädti, A. stellatus, and Huso huso (Detlaf and Ginzburg, 1954). These spaces are formed as a result of the transition of nucleoplasm into cytoplasm during oogenesis after dissolution of the germinal vesicle membrane. In formalin-fixed eggs, the contents of the spaces, like the vesicle contents, are selectively stained bright blue with aniline dye. Cytochemical investigations (Detlaf, 1957; 1961b, 1962) indicate that there are no mucopolysaccharides in the spaces, but the material does contain much glycogen and traces of RNA. When eggs are fixed in Sanfelice fluid, globules of hydrophilic colloid are observed inside the spaces; the nature of the basic component of these globules remains unclear.

Yolk. The sturgeon egg contains numerous yolk granules and lipid inclusions, varying greatly in size; these are small in the animal pole and much larger in the vegetal pole, especially the central part (Figures 12, 14).

In fixed eggs of Acipenser stellatus and A. güldenstädti the yolk granules in the animal pole are generally oval or elongated and do not exceed 2—3μ in diameter; regularly ellipsoid yolk granules, 13—15μ in diameter, predominate in the vegetal pole (Ginzburg, 1956, unpublished).

The large yolk granules of a freshly hatched sturgeon are usually rectangular or square with rounded corners and are up to 30μ long (Grodziński, 1963).

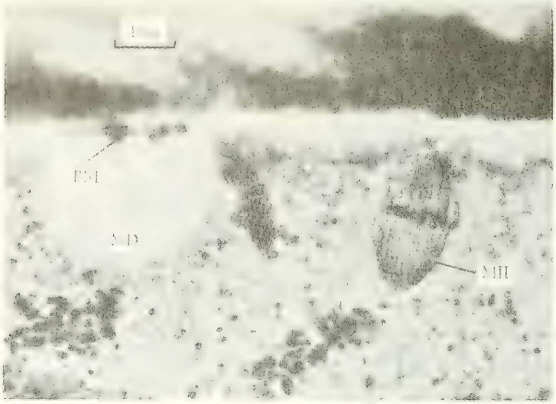

FIGURE 13. Section of mature unfertilized sturgeon egg in area of mitotic figure of second maturation division:

MD — maturation depression; M II — metaphase of second maturation division; PB I — first polar body.

Sturgeon yolk granules have a complex structure. Research on changes in various media, performed on *Acipenser güldenstädti colchicus* (Grodziński, 1963), indicated that the thin outer layer of the granule consists of firm, rather inelastic material and has properties of a semipermeable membrane. When granules are placed in a hypotonic NaCl solution, a trypsin solution or iodine vapor, the core separates into individual plates perpendicular to the long axis of the rectangular granule. According to Grodziński, several of the plates dissolve rapidly; between these are up to ten firmer plates which disintegrate into small grains in a trypsin solution. The patterns observed on dissolution of sturgeon yolk granules resemble similar modifications of selachian (pp. 48—49) and amphibian (Holtfreter, 1946) yolk inclusions.

An ultramicrograph of yolk granules in the animal pole of a stellate sturgeon egg (Ginzburg, unpublished data) clearly showed a dense central core surrounded by lighter material of granular structure; often the outer membrane covers only part of the granule (Plate III). Higher magnification (Plate IV) reveals a system of parallel lines at distances of 50—60 Å in the central core; at an angle of 80° to these lines, another system of distinct lines can be distinguished (Plate IV, b, see arrows). The regular arrangement testifies to the crystalline structure of the central core, similar to that described for the yolk granules of many other animals, including gastropods, leeches, mosquitos, the cyprinid *Danio* and a number of amphibians (see Favard and Carasso, 1958; Karasaki, 1963; Aizenshtadt, 1965; K. Yamamoto and Oota, 1967).

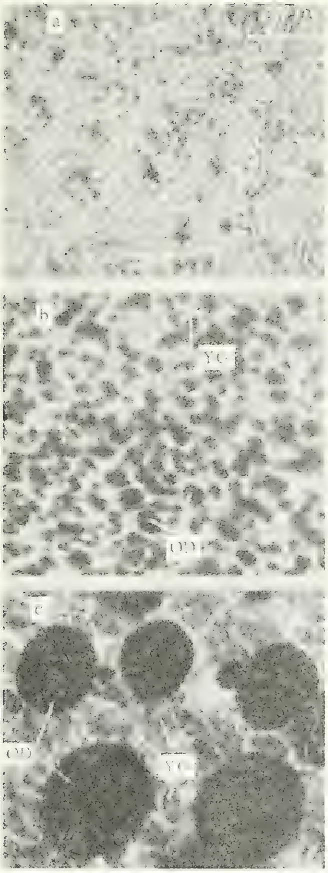

FIGURE 14. Yolk granules in sturgeon egg. The figure shows sections of the animal pole (a), marginal zone (b) and vegetal pole (c):

Osmium treatment in Fleming's fluid. YG — yolk granules; OD — oil droplets.

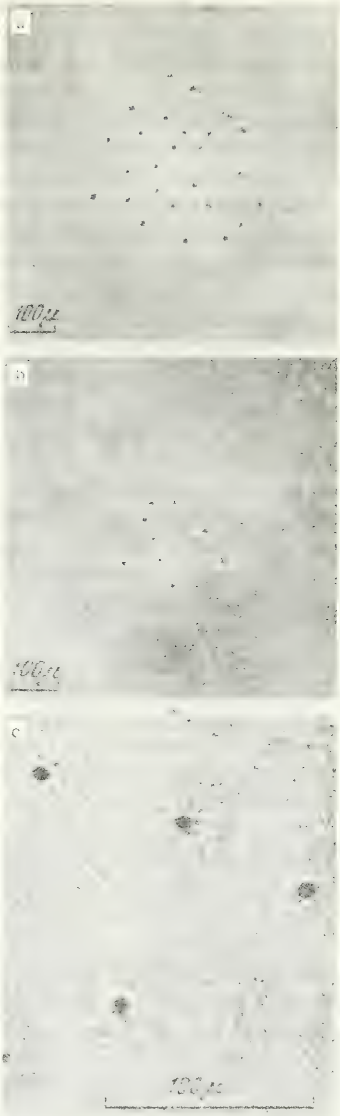

FIGURE 15. Afferent apertures of micropylar canals in eggs of *Acipenser güldenstädti* (a, c) and *Huso huso* (b)

Often there are yolk granules with several fused core parts of different crystalline orientation (Plate IV, a). Within a single mass, the crystalline structure of the material is uniform; there were no signs of different alternating types of plates, as described by Grodziński from observations of patterns of dissolution of the yolk grains.

According to Grodziński's data, sturgeon yolk granules consist mainly of proteins and water; they apparently contain no neutral fats since fatty solvents do not change their shape and Sudan III stains them weakly and varyingly (Grodziński, 1963). However, since they stain gray-blue with Sudan black B (Ginzburg, 1956), they may be assumed to contain phospholipids.

The lipid inclusions in the animal part of Acipenser stellatus and A. güldenstädti eggs are too small to be discerned in the light microscope; if eggs are treated with osmium tetroxide (Figure 14, a) or microscopic sections stained with Sudan black B, a diffuse blackening of the cytoplasm is observed. Ultramicrographs of the surface layer of the egg in this region reveals many liquid inclusions with diameters of 0.5—1.0μ (Plate III). In the vegetal pole, larger lipid inclusions in the form of spherical oil droplets are scattered among yolk granules. In eggs of Acipenser stellatus and A. güldenstädti fixed in formol-calcium, the oil droplets are 2—45μ in diameter (Ginzburg, 1956, unpublished data), in living eggs of A. güldenstädti they are 6—80μ (Grodziński, 1963); cytological tests indicate that they contain neutral fats.

In the egg of Polyodon spathula, as in acipenserids, the yolk grains are larger in the vegetal pole (Larimore, 1950). Members of these families are apparently extremely similar in distribution and consistency of their yolk inclusions, since the arrangement of the cleavage planes in the Polyodon egg is virtually identical to the cleavage pattern of acipenserid eggs (Ballard and Needham, 1964).

Egg membranes. In the mature egg the membranes consist of a well developed two-layered zona radiata and an outer jelly coat, also called the alveolar or honeycomb membrane. The jelly coat of the fertilized egg becomes sticky in water.

The anlage of the zona radiata, in the form of a thin undifferentiated membrane, appears on the surface of young oocytes at the beginning of the growth period. According to certain data (Molchanova, 1941), this occurs before the follicle is formed, but according to other data (Ol'shvang, 1936) it takes place after this. This membrane then thickens and acquires radial striation during vitellogenesis; at the same time, a second membrane is formed on the surface of the zona radiata, consisting of radially arranged columns (the alveolar layer). By the end of oogenesis the zona radiata is divided into two layers, z. r. interna and z. r. externa, which have identical structure (Ol'shvang, 1936; Molchanova, 1941; Votinov, 1947).

According to Sadov's observations (1963), the layers of the zona radiata are not derived from a single membrane but arise independently of one another and at different times.

The later formation of the alveolar layer seems to indicate its secondary origin. Two opposing, unconfirmed opinions were expressed concerning the zona radiata itself. According to one view, this membrane is derived from the oocyte (Ol'shvang, 1936; Molchanova, 1941); according to the other, it is formed by follicular cells (Sadov, 1958, 1963).

The total thickness of membranes in the live unfertilized egg reaches 70μ in Acipenser stellatus and 110—150μ in A. güldenstädti colchicus. In the region of the animal pole, where the micropyles are located, the membranes are less thick — about 50μ in A. stellatus and 80—100μ in A. güldenstädti (Detlaf and Ginzburg, 1954).

According to Sadov (1963), the zona radiata interna is uniformly thick, so that the differences in total thickness of the membranes near the animal pole and in the vegetal pole are due to variations in the zona radiata externa and, particularly, in the gelatinous membrane.

Trypsin affects the integrity of the membranes (G. M. Ignat'eva, unpublished), indicating that they contain proteins. In addition, the membranes contain neutral polysaccharides (Detlaf, 1957); they show a positive periodic acid-Schiff reaction when egg sections are treated with diastase and hyaluronidase, and do not display metachromasy when stained with toluidine blue.

TABLE 9. Number of micropyles in sturgeons

Species	Number of females from which eggs were taken	Number of eggs studied	Number of micropyles in egg			Distribution of eggs of different females, according to average number of micropyles						
			minimum	maximum	average	less than 5	5—10	10—15	15—20	20—25	25—30	30—35
Acipenser stellatus	10	398	1	13	4.8	6	4					
Huso huso	8	140	1	17(33*)	6.6	2	4	2				
Acipenser güldenstädti colchicus	24	972	1	43(52*)	9.7	3	15	2	2		1	1

* This number was found in only one egg.

Micropyle. The egg membranes of sturgeons are furnished with several, sometimes numerous, micropyles (Ginzburg, 1959, unpublished data) (Figure 15). The variation in the number of micropyles in Acipenser stellatus, Huso huso and A. güldenstädti colchicus is very great (Table 9). The degree of variability is generally much less in individual batches of eggs. Among the batches studied, the number of micropyles in individual females varied within limits of 2—10, 3—11, 5—18, 8—28, 10—24, 21—43, etc. Considering the distribution of the number of micropyles in the eggs of single females (Table 9), it appears that in A. stellatus an average number of less than 5 micropyles predominates, and from 5 to 10 in Huso huso and A. güldenstädti. In particular batches of the latter species the average number of micropyles exceeded 25. For A. sturio 3—9 micropyles have been recorded (Dean, 1895, 1896a) and 5—13 for A. ruthenus (Kovalevskii et al., 1870; Zalenskii, 1878; Persov, 1957).

The micropylar apertures are distributed in no particular order over a small area in the region of the animal pole. In A. stellatus, Huso huso and A. güldenstädti colchicus the distance between neighboring apertures usually is from 40 to 80μ, less often up to 100μ.

When there are 5–10 micropyles, these are all located in an area 120–320μ in diameter. In A. güldenstädti eggs with a large number of micropyles (about 30 or more) the outer apertures are sometimes 180–200μ from their neighbors, and the total diameter of the area reaches 1,000–1,100μ.

The individual micropyle is a crateriform pit in the jelly coat, passing into a canal (Figure 6, a). The canal dilates, forming an ampulla from which emerges a narrow terminal tubule whose diameter only slightly exceeds the width of the rod-shaped sperm head. The terminal tubule penetrates the zona radiata interna and reaches the egg surface.

In the region of the micropyle of the unfertilized egg, the cortical layer of cytoplasm forms a protrusion which extends into the terminal tubule, filling its lower part. If an unfertilized sturgeon egg is plamolysed in sucrose or NaCl solution, the cytoplasm separates from the zona radiata interna, and cylindrical extrusions, which apparently filled the terminal portions of the micropylar canals are seen in the region of the micropyle (Ginzburg, 1959).

1.2.5. Holostean Fishes

Holostean fishes (subclass Teleostomi, superorder Holostei) — the bowfin (Amia calva) and gars of the genus Lepidosteus — resemble chondrosteans in manner and conditions of reproduction as well as in shape, size and outward appearance of eggs. Studies of the last century were devoted mainly to the reproductive biology, the external changes of the developing embryo and to the basic morphogenetic processes — cleavage, gastrulation, etc. — in connection with the position of this group in the system (Balfour and Parker, 1882; Fülleborn, 1894; Dean, 1896a, b; Whitman and Eycleshymer, 1897; Eycleshymer, 1899; Ziegler, 1900). Thus, the gametogenesis of this interesting group did not attract attention, and no special cytological research on the eggs of holosteans was undertaken.

The egg is ellipsoidal. In the bowfin the egg is more elongated than in gars, having a diameter of 2.2 × 2.8 mm (Dean, 1896b), or, in terms of limits, 2.0–2.5 × 2.5–3.0 mm (Whitman and Eycleshymer, 1897). Bowfin eggs are grayish brown and those of Lepidosteus osseus yellowish; the animal pole, occupying about a quarter of the egg circumference, has much lighter pigmentation than the yolk-rich vegetal pole.

In internal organization, holostean eggs are apparently also similar to those of sturgeons. The vegetal pole is filled with coarsely granular yolk; in the marginal zone the size of the yolk granules decreases sharply. The similar distribution of the cellular constituents is expressed in the great similarity in pattern of the first cleavage divisions; the pictures of variation in distribution of furrows of the third division produced by Dean (1896b) for the bowfin correspond fully with variations in the cleavage pattern of sturgeons.

Features of the distribution of the cleavage furrows indicate that the concentration of yolk inclusions is higher in holosteans than in sturgeons (see Figure 18).

In the bowfin, cleavage is still complete (Dean's affirmation in the above-cited work concerning the meroblastic nature of cleavage of Amia eggs is erroneous). However, irregularity of cleavage is more sharply expressed in this case and there are already signs of a transition to incomplete cleavage (Whitman and Eycleshymer, 1897; see Ziegler, 1900). The furrows of the first three cleavage divisions reach the vegetal pole and gradually divide the entire yolk-rich vegetal pole, but furrows of the following divisions do not extend beyond the limits of the animal pole. By the end of cleavage there are a large number of small blastomeres in the animal pole bordering on the very large polygonal vegetal blastomeres.

In gar, the yolk concentration is still greater and cleavage is incomplete. The furrows of the first cleavages only reach the egg equator and extend only slightly into the yolk; as a result, the greater part of the vegetal pole remains undivided (Eycleshymer, 1899).

The holostean egg membranes consist of a strongly developed zona radiata surrounded by a sticky villous membrane which aids in attaching the spawned eggs to the underwater parts of plants and other substrates. The surface of the zona radiata in the gar egg is covered by villi with closely packed pyriform free ends. Some investigators consider these villi modified follicular cells (Balfour and Parker, 1882), while other data indicate that they originate prior to the zona radiata and are of primary origin (Mark, 1890). In the bowfin the villi are less dense; in the attached egg, the expanded villi heads adhere to the substrate and the villi are considerably stretched (Dean, 1896b).

One micropylar aperture is located in membranes of the animal pole (Mark, 1890; Whitman and Eycleshymer, 1897).

1.2.6. Teleosts

Structural features and spawning of eggs in relation to developmental conditions. In teleosts (subclass Teleostomi, superorder Teleostei), which include the overwhelming majority of contemporary fishes, conditions of fertilization, spawning and development are extremely diverse. Most fishes of this group, like primitive teleosts (lungfishes, chondrosteans and holosteans), eject gametes into water where fertilization occurs. Eggs of spawning teleosts may be divided into two main groups: pelagic eggs, which develop in midwater, and benthic or demersal eggs.

Mainly marine spawners have pelagic eggs, which are usually small and develop in the upper water layers; large bathypelagic eggs developing at considerable depths are rarer. The reduced specific weight of pelagic eggs, ensuring their buoyancy, is due mainly to the strong hydration of the yolk and, partially, to accumulation of fats. These eggs usually develop individually, but in some species (of the Scorpaenidae, Lophiidae and Antennariidae) there are long, ribbonlike or variously shaped egg masses. The egg mass of Lophius piscatorius can reach tens of meters in length and a meter in width, and may contain several million eggs of 2.1—2.4 mm diameter (Bertin, 1958a). In the rockfish Sebastolobus altivelis, the egg mass is bilobate, each lobe consisting of a complete

TABLE 10. Sizes of teleost eggs

Species	Conditions of egg development	Diameter of egg in membranes, mm	Author
A. Fishes spawning in freshwater			
Gymnarchus niloticus	Demersal, in floating nest	10	Budgett, 1901
Oncorhychus tschawytscha	Demersal, in gravel and sand nest	7–9	Riddie, 1917
Oncorhynchus keta		7–8	Soin, 1956
Oncorhynchus masu		6.5–7.0	Soin, 1956
Salmo trutta m. lacustris		5.3–6.0	Berg et al., 1949
Salmo salar		5.0–6.0	Berg et al., 1949
Thymallus thymallus	Demersal	3–4	Berg et al., 1949
Silurus glanis	Demersal, on plants, defended by male	3.5	Kryzhanovskii, 1949
Esox lucius	Demersal	2.5–3.0	Kazanova, 1954
Coregonus lavaretus	Demersal	2.2–3.2	Kyle and Ehrenbaum, 1929
Coregonus albula	Demersal	2.0–2.2	Berg et al., 1949
Leuciscus idus	Demersal	1.9–2.3	Kryzhanovskii, 1949
Perca fluviatilis	Demersal, ribbonlike egg masses on submerged objects	1.8–2.0	Kryzhanovskii et al., 1953
Misgurnus fossilis	Demersal	1.7–1.9	Kryzhanovskii, 1949
Abramis ballerus	Demersal, on plants	1.7–1.9	Yurovitskii, 1956
Abramis brama		1.6–1.8	Kryzhanovskii, 1949
Rutilus rutilus caspicus		1.6–1.8	Kryzhanovskii, 1949
Cyprinus carpio		1.5–1.8	Kryzhanovskii, 1949
Carassius carassius		1.5–1.7	Kryzhanovskii, 1949
Gobio gobio	Demersal	1.3–1.5	Kryzhanovskii, 1949
Lucioperca lucioperca	Demersal	1.3–1.4	Berg et al., 1949
Oryzias latipes	Demersal	1.2	T. Yamamoto, 1944a
Acerina cernua	Demersal, on plants	1.1–1.2	Kryzhanovskii et al., 1953
Tinca tinca		1.0–1.2	Berg et al., 1949
Lota lota	Demersal	1.0–1.1	Berg et al., 1949
Osmerus eperlanus	Demersal	0.9–1.0	Kazanova, 1954
Nemachilus dorsalis	Demersal	0.9–1.0	Kryzhanovskii, 1949
Cottus kessleri	Demersal, defended by male	0.7–0.9	Berg et al., 1949
B. Fishes spawning in salty and brackish waters			
Bagre marinus	In mouth of male	19–21	Gudger, 1918
Arius commersoni		18	Ihering, 1888
Galeichthys felis		14–17	Merriman, 1940
Lycodes reticulatus macrocephalus	Demersal	8.7	Rass, 1941
Anarhichas latifrons	Demersal	7–8	Berg et al., 1949
Anarhichas lupus	Demersal	5.5–6.0	Berg et al., 1949
Reinhardtius hippoglossoides	Bathypelagic	4.0–4.5	Pertseva-Ostroumova, 1961
Hippoglossus hippoglossus	Bathypelagic	2.9–3.8	Pertseva-Ostroumova, 1961
Belone belone	Demersal	3.1–3.5	Kazanova, 1954
Argentina silus	Bathypelagic	3.0–3.5	Kyle and Ehrenbaum, 1929
Exocoetus volitans	Pelagic	2.7–3.0	Kovalevskaya, 1964

TABLE 10 (Continued)

Species	Conditions of egg development	Diameter of egg in membranes, mm	Author
Cyclopterus lumpus	Demersal, defended by male	2.2–2.6	Kazanova, 1954
Lophius piscatorius	Pelagic	2.1–2.4	Vodyanitskii, 1930
Cottus gobio	Demersal, defended by male	2.0–2.5	Kazanova, 1954
Platessa platessa	Pelagic	1.7–2.6	Pertseva, 1936
Gasterosteus aculeatus	In nest, defended by male	1.6–1.9	Kazanova, 1954
Eleginus navaga	Demersal	1.4–2.1	Rass, 1941
Trigla gurnardus	Pelagic	1.4–1.7	Kazanova, 1954
Clupea harengus pallasi	Demersal	1.3–1.7	Kryzhanovskii, 1956
Sprattus sprattus balticus	Pelagic	1.2–1.7	Kazanova, 1954
Gadus morhua	Pelagic	1.1–1.7	Pertseva, 1936
Clupea harengus membras	Demersal	1.1–1.4	Kryzhanovskii, 1956
Scomber scombrus	Pelagic	1.0–1.4	Berg et al., 1949
Gadus virens	Pelagic	1.0–1.2	Pertseva, 1936
Molva molva	Pelagic	1.0–1.1	Pertseva, 1936
Thunnus thynnus	Pelagic	1.0–1.1	Berg et al., 1949
Rhombus maximus	Pelagic	0.9–1.2	Kazanova, 1954
Trachinus draco	Pelagic	0.9–1.1	Vodyanitskii, 1930
Ammodytes tobianus	Demersal	0.8–1.0	Kazanova, 1954
Pleuronectes flesus	Pelagic	0.8–1.0	Pertseva, 1936
Mullus barbatus	Pelagic	0.8–0.9	Vodyanitskii, 1930
Limanda limanda	Pelagic	0.6–1.0	Berg et al., 1949
Coris julis	Pelagic	0.6–0.7	Vodyanitskii, 1930
Gobius niger	Demersal	0.5	Kryzhanovskii and Pchelina, 1941
Gobiosoma bosci	Demersal	0.5	Kuntz, 1916
Ctenogobius stigmaticus	Demersal	0.3	Kuntz, 1916
C. Viviparous			
Belonesox betizanus		4.1	Scrimshaw, 1946
Zoarces viviparus		About 3	Berg et al., 1949
Poecilia (Lebistes) reticulata		2.0–2.1	Scrimshaw, 1946
		1.8–2.0	Ginzburg, inpublished
Xiphophorus helleri		1.9–2.1	
Gambusia affinis holbrooki		1.6	Scrimshaw, 1946
Sebastes marinus		1.1–1.7	Kyle and Ehrenbaum, 1930
Aulophallus elongatus		0.6	Scrimshaw, 1946
Heterandria formosa		0.4	Scrimshaw, 1946

conical case (up to 60 cm long) in which eggs, 1.2—1.4 mm in diameter, are arranged in a single layer glued by gelatinous substance of albuminous consistency (Pearcy, 1962).

In species spawning in freshwater, pelagic eggs are exceptional. One reason for this difference between marine and freshwater eggs is the fact that freshwater eggs must have much lower specific weight in order to maintain buoyancy. The pelagic eggs of Clupeonella delicatula, which breeds in brackish and freshwater, are buoyant due to their very large oil inclusion and the large size of the perivitelline space. Some tropical fishes spawning in small stagnant water bodies containing oxygen only at the surface (Polyacanthus, etc.) have pelagic eggs with a very large oil drop (Kryzhanovskii, 1960).

The eggs of shad, Pelecus cultratus, many cyprinids of the Amur and Siniperca chua-tsi develop in midwaters of rivers, but settle on the bottom in standing waters; these are polypelagic eggs. Such eggs are usually buoyed up by strong swelling of the outer membrane and formation of a large perivitelline space (Nikol'skii, 1948; Kryzhanovskii, 1960).

Demersal eggs are found mainly in freshwater fishes and in marine fishes spawning in the littoral. In some fishes, e.g., in Cottidae, Cyclopteridae, some Blenniidae and Anarhichadidae, the eggs are joined in masses. Perch eggs are joined by a gelatinous substance into broad reticulate ribbons or cylinders up to 2—3 m long, attached to submerged plants (Kryzhanovskii et al., 1953; Bertin, 1958a). Flying fishes of the genus Exonautes have egg membranes with long filiform outgrowths which attach the eggs to sargasso weeds and join them in large concentrations over half-a-meter in diameter, these concentrations were long thought erroneously to be floating "nests" of other fishes from the Antennariidae family (Gudger, 1937).

Most demersal eggs develop individually. Some eggs are distributed on the bottom, and not attached to any substrate (salmon, Alosa, burbot), but in most cases demersal eggs have stickly membranes which adhere to stones, sand, plants, etc. (cyprinids, pike, Clupea, loaches and many others).

Apart from pelagic and demersal eggs, there are groups in which eggs develop under special conditions created by various adaptations for care for progeny: in nests, in the mantle of bivalve mollusks, under the carapace of a crab, in the mouth of parents, in special pouches or brood cases developing in the male (see Bertin, 1958b; Lagler et al., 1962).

Finally, three teleost orders exhibit internal fertilization and viviparity in which eggs of various species develop in the follicle or ovarian cavity of the female. Live-bearers are found among many cyprinodonts (Poeciliidae, Goodeidae, Jenynsiidae and Anablepidae families), some Perciformes (Embiotocidae, Clinidae, Zoarcidae, Brotulidae, Scorpaenidae, Cottidae, and Blenniidae families) and one family of Beloniformes — the Hemiramphidae (see Eggert, 1929; Turner, 1947; Bertin, 1958c; Amoroso, 1960).

The structure of the eggs, embryo and larvae with respect to conditions of development have been summarized in detail by Kryzhanovskii (1948, 1949, 1956, 1960; Kryzhanovskii et al., 1951). Among fishes of Soviet waters, Kryzhanovskii distinguishes the following groups (according to manner of reproduction and spawning): lithophilous (eggs deposited on sandy-stony bottom), phytophilous (eggs attached to plants), psammophilous (eggs develop on open sandy bottom after spawning), pelagophilous (development in midwater), ostracophilous (eggs spawned in mantle cavities of bivalve mollusks). Eggs and embryos of species belonging to each of these groups have characteristic adaptational structural traits.

Egg shape. The overwhelming majority of teleost eggs are spherical in shape. Various deviations from this shape are shown in Figure 16. Slight deviations are observed quite often among the Perciformes; thus, elliptical eggs are found in some species of Scorpaenidae, Dactylopteridae, Ammoditidae, Gobiesocidae, Blenniidae and Carapidae families, elliptical, ovate or irregularly shaped eggs in Cichlidaema, elliptical or cylindrical eggs with rounded ends in Pomacentridae (Breder, 1943). Among Clupeiformes, most species of Engraulidae produce elliptical eggs, while some produce drop-shaped eggs (Vodyanitskii, 1930; Delsman, 1930; Breder, 1943). Among the Siluroidei, elliptical eggs are described in some Ariidae (Gudger, 1918; Merriman, 1940). In Cypriniformes, only the eggs of *Rhodeus* (Cypriniidae), which spawns in the mantle cavity of bivalve mollusks, are variously elliptical, elongate, and sometimes pointed (Kryzhanovskii, 1949). The most substantial deviations from the spherical shape are found among eggs of Gobiidae (order Perciformes), whose shape varies very considerably (Breder, 1943); spherical, elliptical, drop-shaped, pear-shaped, cylindrical and sometimes highly distinctive. After the egg of *Bathygobius soporator* enters the water, its membrane assumes an unusual shape resembling a coffin (Figure 16, no. 17); this membrane shape (like the egg capsule of chimaeras) is adapted to the size and organization of the embryo in the later stages of development (Tavolga, 1950).

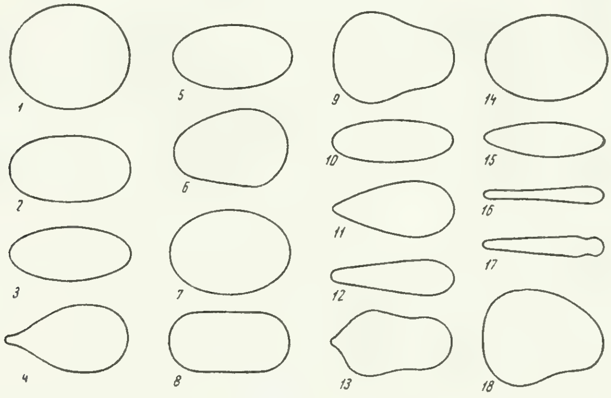

FIGURE 16. Nonspherical teleost eggs:

1–4 — eggs of anchovy (1 — *Anchoviella mitchilli*; 2 — *Engraulis capensis*; 3 — *E. japonicus*; 4 — *Stolephorus indicus*); 5 — egg of cyprinid (*Rhodeus amarus*); 6 — egg of cichlid (*Tilapia macrocephala*); 7, 8 — eggs of Pomacentridae (7 — *Chromis chromis*; 8 — *Pomacentrus leucostictus*); 9–18 — eggs of Gobiidae (9 — *Gobius microps*; 10 — *Gobiosoma bosci*; 11 — *Gobius ophiocephalus*; 12 — *Gobius niger*; 13 — *Gobius minutus*; 14 — *Gobius* sp.; 15 — *Gobius nudiceps*; 16 — *Glossogobius brunneus*; 17 — *Bathygobius soporator*; 18 — *Gobius flavescens*) (after Breder, 1943). All the eggs in the drawing are orientated with micropyles facing left.

Egg dimensions. The teleost egg diameter varies greatly, between 0.3 and 20 mm, but is of the order of 1—3 mm in most species. On the average, demersal eggs are larger than pelagic.

Data on egg sizes are given in Table 10, which gives only a general idea of size variability. The data are not entirely comparable, since data have been included on the diameters of developing fertilized eggs (Vodyanitskii, 1930; Pertseva, 1936; Rass, 1941; Kryzhanovskii, 1949, 1956; Kazanova, 1954; Kovalevskaya, 1964), in addition to information on the little known mature unfertilized eggs (e.g., Kuntz, 1916; Merriman, 1940; part of data of Scrimshaw, 1946). Some sources (e.g., Berg et al., 1949) do not indicate whether the eggs are unfertilized or fertilized (see references on fish egg sizes in the articles of Rass, 1941, 1947, 1953 and Zotin, 1961b).

Among fishes spawning in freshwater (Table 10, A) the largest eggs — 10 mm diameter — are those of Gymnarchus niloticus (belonging to the order Mormyriformes having forms closely related to Clupeiformes). Other fishes with large eggs, up to 5—9 mm in diameter, belong to the genera Oncorhynchus and Salmo (Clupeiformes).

Among fishes spawning in seawater (Table 10, B), huge eggs, up to 17—21 mm in diameter, are produced by fishes of the Ariidae family (Bagre marinus, Arius commersoni, Galeichthys felis); these eggs — the largest known for teleosts — are nurtured in the mouth of the male, and in A. commersoni sometimes by the female (Ihering, 1888; Gudger, 1918). Demersal eggs of some Zoarcidae (e.g., Lycodes reticulatus), wolffishes and Beloniformes, as well as some flatfishes are also large. Most marine fishes lay pelagic eggs, 1—2 mm in diameter. The smallest eggs described are those of Ctenogobius stigmaticus inhabiting brackish waters of North Carolina.

Eggs of live-bearing teleosts (Table 10, B), as well as live-bearing Euselachiae, vary considerably in size. For example, in Poecileridae the diameter of Belonesox betizanus eggs reaches 4.1 mm, but is only 0.6 mm in Aulophallus elongatus and 0.4 mm in Heterandria formosa. The reduction of the yolk in the last two species is related to adaptations enabling the embryo to obtain nutrition from the female (Turner, 1947).

Egg size is a stable species character. During the spawning season, the average egg size usually decreases, but the amplitude of size variations hardely changes (Rass, 1947, 1953).

Egg size and external conditions are undoubtedly related. Thus, eggs are larger, on the average, the farther north the distribution area of the species. An increase in egg size is also observed in the direction from shallow to deep waters. In both cases, the increase in size is parallel to decrease in temperature (Rass, 1935; 1941; Marshall, 1953). The reasons for this correlation are not yet fully understood. Lowering of temperature may exert a direct effect on the size of fertilized eggs; it has been shown experimentally that the lower the temperature at which fertilization occurs, the more strongly the eggs swell (see Rass, 1953). However, the increased size in northerly forms is apparently connected with increased yolk reserves and is accompanied by an increase in the size of hatching larvae. A possible adaptational significance of such a change is that larger larvae survive better in conditions of sparse food in deep and cold waters, since large larvae have a lower food requirement (relative to size) and are able to seek food in a larger area (Marshall, 1953). In some species with pelagic eggs, the size is inversely proportional to salinity (see Rass, 1953).

On the one hand, there is correlation between egg size and fecundity. As a rule, the more eggs spawned, the smaller the egg. For example, cod (egg diameter, 1.1—1.7 mm) produces 10 million eggs and burbot (egg diameter, 1.0—1.1 mm) 5 million, whereas Atlantic salmon (egg diameter, 5—6 mm) produces 6,000—26,000 eggs and summer chum (egg diameter, 7—8 mm) 2,000—3,000 (Nikol'skii, 1954); Bagre marinus and Galeichthys felis, which have the largest teleost eggs (diameter, 14—21 mm), spawn no more than 20—40 eggs (Merriman, 1940).

Data on egg size and fecundity, obtained by various authors for a large number of species belonging to various families and orders, have shown (Zotin, 1961b) that there is no regular correlation between egg size and absolute fecundity. However, the relation between egg sizes and relative fecundity of the species, which is defined as the absolute number of eggs per unit weight of the fish, has been established as distinctly logarithmic.

In species whose eggs develop in open waters or are freely scattered on the bottom, population strength is usually maintained by high fecundity relative to the small egg size. Development of adaptations for care of progeny is accompanied by decreased fecundity and usually increased egg size with greatly increased energy resources for the embryo. Thus, the energy reserves of the small pelagic eggs of the Black Sea surmullet are given at 0.045 cal per egg, while the large eggs of the salmon, which develop in gravel and sand nests, have reserves 6,500 times higher, i.e., 305.60 cal per egg (Faustov and Zotin, 1967). Accordingly, increased egg size enables the embryo to develop without obtaining exogenous food, and to reach active life at a higher level of differentiation, ensuring higher survival.

General structure of the egg. In teleost eggs, the concentration of reserve (deutoplasmic) inclusions is so large that they press all or almost all the cytoplasm to the periphery. In the center of the animal pole, the peripheral layer of cytoplasm is thick (expressed more or less distinctly in different fishes), while toward the marginal area this layer gradually thins (Hoffmann, 1881, etc.). This thickening of the peripheral cytoplasm is the anlage of the blastodisc; after fertilization the yolk-free cytoplasm shrinks toward the animal pole, and the thickening increases and turns into a convex blastodisc. In the sharply telolecithal egg, cleavage furrows only divide the blastodisc and cleavage is incomplete (discoidal).

A layer of cortical alveoli lie in the surface cytoplasm of the mature unfertilized egg. The nucleus at metaphase of the second maturation division is to be found in the animal pole close to the micropyle.

The greater (central) part of the egg contains yolk inclusions. Protein yolk inclusions in some species remain disorganized and dispersed in the cytoplasmic stroma as separate granules; in other species they fuse into a single homogeneous mass and many fishes also have oil droplets (see pp. 73, 75).

The perch egg shall serve as the example of teleost egg structure here (Thomopoulos, 1953a; cf. Figure 17). The entire central part of the mature perch egg is filled with a homogeneous yolk mass. The peripheral cytoplasm is about 40μ thick on the average, and distinctly thicker at the animal pole. This layer contains a few small yolk granules (not exceeding 5μ in diameter) and an uninterrupted row of cortical alveoli, about 20μ in diameter, at the surface (in the diagram of Figure 17 they appear to be a certain distance apart, but are actually close together). In the yolk, close to the animal pole, there is a large oil drop; around this there are often several small oil droplets. At the transition between the cytoplasm and yolk, the cytoplasm is mixed with yolk or else combines with it. The egg has two membranes: the inner — zona radiata — is about 6μ thick, while the outer jelly coat is about 50μ thick (see Flügel, 1967).

Different fishes show different ratios between the amount of yolk and the yolk-free cytoplasm. This difference is particularly marked after fertilization, when the blastodisc is already fully formed, and in cleavage stages (cf. Figure 18). The salmon can serve as an example of yolk-rich eggs; thus the egg diameter in Salmo salar is 5.5—6.0 mm and the diameter of the blastodisc prior to the first cleavage is 1.2—1.3 mm (Battle, 1944), so that the area of the blastodisc in contact with the yolk is 1% of the egg surface. A complete series of transitions with gradual decrease in yolk content can be demonstrated, right up to the very small eggs of gobies in which the cytoplasm equals or sometimes even exceeds the amount of yolk (Ctenogobius stigmaticus — Kuntz, 1916; Gobius niger, Gobionellus — Kryzhanovskii and Pchelina, 1941). In these species the relative size of the blastodisc is several dozen times greater than in salmon.

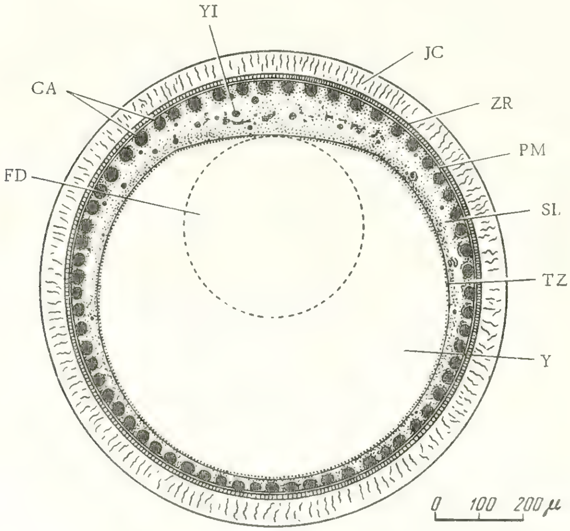

FIGURE 17. Diagrammatic section of mature unfertilized perch egg (after Thomopoulos, 1953a):

Y — yolk; YI — yolk inclusions in surface cytoplasm; FD — oil drop; CA — cortical alveoli; TZ — transitional zone of cytoplasm-yolk; PM — plasmic membrane; SL — surface layer of cytoplasm; JC — jelly coat; ZR — zona radiata.

According to the amount of yolk, teleost eggs may be subdivided conditionally into two main groups: oligoplasmic, containing much yolk and little cytoplasm, and polyplasmic, richer in cytoplasm (Kryzhanovskii, 1940). All large eggs are oligoplasmic, whereas eggs with a diameter of 1.5 mm or less may be oligoplasmic or polyplasmic. Planktonic eggs are all oligoplasmic; demersal eggs may belong to either group.

Coloration. Teleost eggs are usually transparent or semitransparent, either colorless or yellowish, greenish or grayish; egg color is intense only rarely (e.g., in some salmons and bitterlings). A yellow or orange color is due to fat-soluble carotenoids (Goodwin, 1951, 1952) including carotenes (C-carotene), principally, xanthophylls (lutein, astaxanthin, and pigment related to or identical with taraxanthin). Elucidation of the function of carotenoids at different stages of development is still far from satisfactory. The suggested role of astaxanthin in fertilization as a substance activating movement of the spermatozoa (gynogamone I) remained unproved until recently (see p. 163). Study of the morpho-ecological patterns of development in Salmonidae, Clupeidae, Cyprinidae and certain Perciformes (Blennius sphynx, Baikal sculpins) led to the suggestion first made in 1935 by S. G. Kryzhanovskii and then elaborated by his students S. G. Soin and A. I. Smirnov (Smirnov, 1950; Soin, 1962), that the carotenoids have a respiratory function. The suggestion is based on the observation that pigment intensity, which depends on the presence of carotenoids in the egg, is more intense when less oxygen is available. However, this hypothesis has not yet been confirmed biochemically. The established differences in intensity of carotenoid pigmentation in the egg may be connected not only with conditions of respiration, but also with the conditions of excretion of metabolites (Yarzhombek and Grachev, 1964). Data, relating to sea urchins, generally argue against the participation of carotenoids in metabolic processes in the egg, since throughout embryonic development these pigments show no quantitative or qualitative changes (Griffiths, 1966). As in bacteria, carotenoids may protect the cytoplasm from the harmful effects of light in some invertebrate and teleost eggs (similar to the function of melanin in eggs of other animals).

Cortical alveoli are often arranged in a single row, e.g., in chum salmon (K. Yamamoto, 1951b) or lake trout (Ginzburg, unpublished), but sometimes they are so numerous that they lie in two or three rows, as in goldfish (Tchou-Su and Chen-Chao-hsi, 1936). The layer of cortical granules is generally interrupted near the micropyle (in the nuclear region).

The absence of alveoli in this region was established for Oryzias latipes (T. Yamamoto 1939, 1944a), Fundulus (Wessels, 1953), Pacific herring (Kanoh, 1953), sand lance (Thomopoulos, 1954), chum salmon, sokeye, rainbow trout, char, two species of sticklebacks (Kusa, 1956), etc. In goldfish there are isolated alveoli in the region of the spindle (Tchou-Su and Chen-Chao-hsi, 1936). The size of the cortical alveoli varies greatly; in many species a single egg may contain alveoli ranging in diameter from a few to 20 or 30μ. The maximum size of alveoli is 35—40μ (see Table 1).

Protein yolk. This accumulates in the oocytes in the form of yolk granules. In some fishes (e.g., many cyprinids, loach, Macropodus) the yolk granules undergo no visible changes upon maturation of the eggs, but remain isolated formations enclosed in a delicate cytoplasmic stroma.

In other species (herring, bream) the yolk granules begin to merge into large, irregular lumps in oocytes before spawning, when this process is temporarily suspended; it is then terminated soon after fertilization (Hoffmann, 1881; Kolpakova, 1951; M. F. Ivanov and Dodzina, 1957; K. Yamamoto, 1958b). In cisco, whitefish, grayling and pike, the merging of yolk granules on egg maturation occurs later than in clupeids, most granules unite, although some are still isolated. Finally, complete union of the granules occurs at the end of oogenesis in a large number of species, and the entire yolk becomes a homogeneous mass. Such fishes include many salmonids (chum salmon, salmon, sea trout, lake trout, etc.), pike perch, and, apparently, other Percidae, Cyprinodontidae (egg-laying and live-bearing), Pleuronectidae, turbots, sticklebacks and many others. In particular, complete union of the yolk granules is characteristic for species with pelagic eggs, since such eggs become more transparent and are less noticeable in the water column.

The sizes of yolk granules in certain teleost species are shown in Table 11. Small granules are distributed mainly in the egg periphery, large ones more centrally.

TABLE 11. Sizes of yolk granules in teleost eggs

Family	Species	Diameter, μ	Remarks	Author
Clupeidae	Clupea harengus membras	Up to 50–80		Kupffer, 1878
Salmonidae	Coregonus albula	10–13	The greater part of the yolk granules merge into a single mass	Szubińska-Kilarska, 1959
	Coregonus lavaretus	11–19		
Thymallidae	Thymallus thymallus	12–18		
Esocidae	Esox lucius	16–22		Szubińska, 1961
Cyprinidae	Rhodeus amarus	6–19		Grodzinski, 1954
	Carassius auratus	Up to 30–40		Tchou-Su and Chen-Chao-hsi, 1936
	Cyprinus carpio	6–24		Szubińska, 1961
	Brachydanoi rerio	0.3–30		K. Yamamoto and Oota, 1967
Cobitidae	Misgurnus fossilis	4.5–48		Szubińska, 1961
Percidae	Perca fluviatilis	Up to 25		Meien, 1927
			All granules unite on maturation	Thomopoulos, 1953a
		2.3–29		Szubińska, 1961
	Acerina cernua	4–21		Szubińska, 1961
Anabantidae	Macropodus opercularis	5–13		Grodziński, 1956
	Betta splendens	3–9		Grodziński, 1956

It is a widely-held opinion that the protein yolk inclusions in teleosts are organized in vesicles delimited by membranes and filled with fluid. In accordance with this view, yolk granules have been called "yolk spheres" over a long period (Dotterkugeln—Kupffer, 1878; Hoffmann, 1881; vitelline or yolk globules — Cunningham, 1897; K. Yamamoto, 1958a; Osanai, 1956; yolk spheres — Grodziński, 1954, 1956; Szubińska-Kilarska, 1959; Chopra, 1960; Szubińska, 1961).

However, ultramicroscopic study of Brachydanio rerio (K. Yamamoto and Oota, 1967) has shown that the central body of the yolk granule has, as in a number of other animals, a crystalline structure. The yolk granules in this species are very similar in organization to those of sturgeons (p. 58): the central body often consists of several elements with different orientations of the crystalline network; it is enclosed in granular and light material and is bounded by a membrane, part of which is often fragmentary.

The membrane bounding the yolk granule, like other cytoplasmic membranes, consists of proteins and lipids. This is confirmed by the effect on yolk granules of agents dissolving lipids and coagulating proteins (Grodziński, 1954, 1956; Szubińska-Kilarska, 1959; Szubińska, 1961).

Cytochemical investigations indicate that the basic constituents of yolk granules are also proteins and lipids. The proteins possess acid properties; the lipids are mainly phosphatides (in particular, lecithin) and a small quantity of neutral fats in some species (Konapacka, 1935, 1937; Mas, 1953; Arndt, 1956; K. Yamamoto, 1957b, 1958b; Chopra, 1958b, 1960). Apparently proteins are associated with lipids as lipophosphoproteids (ichthulin). In addition to proteins and lipids, polysaccharides have also been observed in the yolk granules (Ihnuma and Tsukuda, 1952; Mas, 1953; K. Yamamoto, 1956d; Chopra, 1960).

Fatty yolk. Most teleosts have, in addition to protein yolk granules, separate lipid inclusions. In oogenesis, these appear prior to the yolk granules, in some species before and in others after the emergence of vacuoles — the precursors of the cortical granules (see p. 11).

The number and distribution of lipid inclusions in various fishes vary greatly (Agassiz and Whitman, 1885a; Cunningham, 1897; Rass, 1953; Szubińska-Kilarska, 1959; Szubińska, 1961; Kryzhanovskii, 1960). For example, there are no oil droplets in some gadids (Gadinae subfamily, Pleuronectidae, Labridae, many clupeoids (Clupea, Sprattus, etc.) and Cobitidae, so that fatty substances are stored in the protein yolk. In some cyprinids small lipid inclusions have been described; in carp and bitterling, a small quantity of oil drops, 1–1.5 μ in diameter, were found in yolk removed from the egg, but were indiscernible in the intact egg. These most probably result from destruction of the yolk granules (Szubińska, 1961). In the unfertilized pike egg, small oil droplets are situated under the anlage of the blastodisc (Arndt, 1956) In salmonids, grayling and stickleback, numerous oil droplets of various sizes are scattered over the yolk surface below the outer cytoplasmic layer; in whitefish and stickleback such droplets coalesce as the egg develops. In many fishes a similar union occurs during oogenesis, and the mature egg contains one large oil drop. Examples of this type of egg are given in Table 12. Several small oil droplets are often found together with the large drop, but this is not always noted by investigators

TABLE 12. Sizes of oil drops in teleost eggs

Family	Species	Oil drops		Author
		number	diameter, μ	
Clupeidae	Sardina (Clupea) pilchardus	1	160	Kyle and Ehrenbaum, 1929
	Clupeonella delicatula	1	320–400	Kryzhanovskii, 1956
Salmonidae	Salmo trutta trutta	Many	20–260	Grodziński, 1949
	Coregonus albula	17–20	14–330	Szubińska-Kilarska, 1959
	Coregonus lavaretus	Many	14–264	Szubińska-Kilarska, 1959
Thymallidae	Thymallus thymallus	Many	10–250	Szubińska-Kilarska, 1959
Argentinidae	Argentina sphyraena	1	370–470	Kyle and Ehrenbaum, 1929
	Argentina silus	1	950–1160	Kyle and Ehrenbaum, 1929
Esocidae	Esox lucius	Many	10–60	Szubińska, 1961
Cyprinidae	Cyprinus carpio	Many	1–1.5	Szubińska, 1961
Gadidae	Molva molva	1	280–310	Pertseva, 1936
	Brosmius brosme	1	230–320	Pertseva, 1936
	Motella argentea	1	150	Agassiz and Whitman, 1885b
	Lota lota	1	430	Kryzhanovskii, 1960
Serranidae	Serranus scriba	1	120–150	Kryzhanovskii et al., 1953
Percidae	Lucioperca lucioperca	1 (+ several small)	500–700	Kryzhanovskii et al., 1953
	Perca fluviatilis	1	440–710	Kazanova, 1954
		1 large (+ several small)	410–413 (5–27)	Szubińska, 1961
	Acerina acerina	1	600–650	Kryzhanovskii et al., 1953
	Acerina cernua	1	390–450	Kryzhanovskii et al., 1953
		1 large (+ several small)	421–463 (3–40)	Szubińska, 1961
Carangidae	Trachurus trachurus	1	230–280	Vodyanitskii, 1930
Mullidae	Mullus barbatus	1	160–250	Kryzhanovskii et al., 1953
Trachinidae	Trachinus draco	1	190–230	Vodyanitskii, 1930
Ammoditidae	Ammodytes tobianus	1 large (+ several small)	240	Thomopoulos, 1954
Scombridae	Scomber scomber	1	250–350	Kyle and Ehrenbaum, 1929
Triglidae	Trigla gurnardus	1	190–330	Kyle and Ehrenbaum, 1929
Bothidae	Rhombus maximus	1	150–220	Kyle and Ehrenraum, 1929
	Paralichthys olivaceus	1	130–190	Pertseva-Ostroumova, 1961
Cottidae	Myoxocephalus (Cottus) scorpius	1	400–500	Kazanova, 1954
Agonidae	Agonus cataphractus	1	700–750	Kazanova, 1954
Lophiidae	Lophius piscatorius	1	400–420 530–570	Agassiz and Whitman, 1885b Kyle and Ehrenbaum, 1929

when describing egg structure. The absolute size of a single oil drop varies greatly, from 0.12 to 1.16 mm in species mentioned in the table.

The relative size of the oil drop (ratio between diameter of drop and that of entire yolk) is usually greater in freshwater fishes than in marine fishes. For example, among perches, the relative size varies between 0.16 and 0.29, and between 0.44 and 0.66 in freshwater fishes. In some freshwater pelagic eggs, the oil drop has an enormous relative size, e.g., 0.76 in Clupeonella delicatula and 0.80 in the tropical fish Polyacanthus sp. (Kryzhanovskii et al., 1953).

The oil droplets in fish eggs have a double function: they store nutritive substances of high calorie value and have a hydrostatic effect due to their low specific density (Kryzhanovskii, 1960). Their role in ensuring buoyancy of pelagic eggs has already been mentioned. The position of the large oil drop differs in marine and freshwater eggs. In the former, it is usually in the vegetal hemisphere, so that the developing egg is orientated with the blastodisc downward. In later stages of embryonic development, the oil drop enters the posterior part of the yolk sac; due to this, active larvae rapidly sink to deeper and calmer water layers, while passive larvae remain at a definite level or rise to the surface. In freshwater fishes (e.g., perches, burbot) the oil drop is located in the animal hemisphere and later enters the anterior part of the yolk sac; because of these movements, the larva floats to the upper water layers where aeration is more favorable.

The fatty inclusions contain mainly neutral fats (glycerides). In many species, these are apparently mixed with phosphatides, which precipitate in the form of crystals under certain conditions. No proteins were found in the oil drops (Fauré-Fremiet and Garrault, 1922a, c; Grodziński, 1949, 1956; Mas, 1953; K. Yamamoto, 1958a; Szubińska-Kilarska, 1959; Chopra, 1960; Szubińska, 1961). In eggs of Coregonus albula lucinensis, there are two types of lipid inclusions: the greater part of the drops contain phosphatides, the smaller part neutral fats (Arndt, 1956).

In addition to protein and fatty yolk inclusions, teleost eggs contain ancillary substances in the form of glycogen lumps. In carp and gudgeon (Konopacka, 1935), glycogen accumulates only at the end of oogenesis. In the flatfish Liopsetta obscura (K. Yamamoto, 1956c) and Vimba vimba (Sakun, 1960), glycogen is found from the early stages of oogenesis.

Egg membranes. The zona radiata is generally single-layered. In some fishes — chum salmon (Kanoh and Yamamoto, 1957), Baltic herring (M F. Ivanov, 1958), Atlantic herring (Hoffmann, 1881), Pacific herring (T. S. Yamamoto, 1958), perch (Flügel, 1967), Salarias flavo-umbrinus (Eggert, 1929) and others — it is two-layered as in sturgeons. In the South American cyprinidonts Pterolebias longipinnis and two species of Cynolebias, polarized light reveals an even more complex organization in this membrane in which 6—8 layers are discernible; such structural complications, may be assumed to be associated with adaptation to survival in bottoms of drying water bodies (Siegel, 1957/1958).

The basic substance of the zona radiata is homogeneous and pierced by tubules which open on the surface through pores. In specimens studied the diameter of these pores is generally less than 1 μ and the distance between them varies in different species from 0.6 to 2.4 μ (Table 13). The

TABLE 13. Thickness and structure of zona radiata in teleosts

Family	Species	Thickness of zona radiata, μ	Diameter of pore, μ	Distance between pores, μ	Author
Clupeidae	Clupea harengus harengus	32.5			Hoffmann, 1881
	Caspialosa kessleri pontica	6.6			Zotin, 1953
	Caspialosa volgensis	2–3			Khaldinova, 1940
Salmonidae	Salmo salar	46–57		1.5–2.0	His, 1873
	Salmo salar m. sebago	43			Zotin, 1953
	Salmo trutta m. lacustris	30–35			Blanc, 1894
		38			Zotin, 1953
	Salmo trutta m. fario	33–37		1.37	Becher, 1928
	Salvelinus alpinus	25–26		1.7	Becher, 1928
	Coregonus fera	15–16		2.0–2.4	Becher, 1929
	Coregonus lavaretus baeri	29			Zotin, 1953
Osmeridae	Osmerus eperlanus	13.32			Sadov, 1963
Cyprinidae	Alburnus alburnus	4.4			Zotin, 1953
	Abramis brama	9			Zotin, 1953
	Blicca bjoerkna	5.7			Zotin, 1953
	Vimba vimba vimba carinata	10			Zotin, 1953
	Tinca tinca	4.4			Zotin, 1953
	Gobio gobio	7.1			Sadov, 1963
Cobitidae	Cobitis taenia	9.7			Zotin, 1953
Cyprinodontidae	Pterolebias longipinnis	24			Siegel, 1957/58
	Cynopoecilus splendens	14			Siegel, 1957/58
Melanotaeniidae	Melanotaenia maccullochi	5			Sterba, 1957
Percidae	Perca fluviatilis	6.66			Sadov, 1963
	Acerina cernua	13.3			Sadov, 1963
Carangidae	Trachurus trachurus	5.9			Zotin, 1953
Cichlidae	Hemichromis bimaculatus		0.41–0.62	1.25	Wickler, 1956
	Cichlasoma festivum		0.41–0.62	1.25	Wickler, 1956
	Pterophyllum scalare		0.31–0.5	1.12–1.37	Wickler, 1956
	Biotodoma (Geophagus) cupido		0.21–0.75	1.25	Wickler, 1956
	Etroplus suratensis		0.38–0.51	1.54(–2.31)	Wickler, 1956
Labridae	Julis vulgaris	8			Hoffmann, 1881
Blenniidae	Blennius fluviatilis (vulgaris)		2 types of pores: 0.3–0.4 1.25(–2.5)	0.6–0.8	Wickler, 1957
	Salarias flavo-umbrinus	5–6.5			Eggert, 1929
Maenidae	Spicara smaris flexuosa	18.8			Zotin, 1953
Soleidae	Solea lascaris	5.9			Zotin, 1953
Syngnathidae	Syngnathus nigrolineatus	2.4			Zotin, 1953

thickness of the zona radiata varies considerably, from 2–3 μ in *Caspialosa volgensis* and *Syngnathus nigrolineatus* to 30–38 μ in trout and 43–57 μ in salmon.

As already noted (p. 35), the outer gelatinous or villous membrane in demersal eggs is extremely varied in structure.

There is a single micropyle in the region of the animal pole of teleost eggs. It is a funnel-shaped pit in the membrane surface, passing into a short terminal tubule which opens into the cytoplasm at the inner surface of the zona radiata (usually at a small protuberance of this membrane into the cortical layer, cf. Figure 6).

The diameter of the micropyle tubule corresponds to the width of the spermatozoon head of the particular species (Table 14). Thus, no more than one spermatozoon can advance at a time along the micropylar canal. This was first noted in the case of salmon by His (1873), who stressed its significance in fertilization.

Histochemical investigations indicate that the zona radiata of some fishes — Pacific herring (T. S. Yamamoto, 1957), char (Osanai, 1959), *Oryzias latipes* (T. S. Yamamoto, 1955), *Leignathus argenteum* (Ihnuma and Tsukuda, 1952) — contains proteins and neutral polysaccharides. In cyprinids the zona radiata and villous layer are purely protein; between these membranes there is a thin polysaccharide-rich layer of primary origin (Arndt, 1960c).

The proteins composing the zona radiata are soluble in water and in salt solutions. In salmonids (trout, salmon, chum salmon) they resist the effect of pepsin and trypsin (Bourdin, 1926; Young and Inman, 1938; Hayes, 1942; Kanoh and Yamamoto, 1957). The egg membrane of chum salmon is lysed when treated with acidified Ringer's solution and then with pancreatin (Kanoh and Yamamoto, 1957). In other fishes (*Oryzias latipes*, Pacific herring, loach) the membrane is lysed by proteolytic enzymes (Ishida, 1944; T. S. Yamamoto, 1958; Kostomarova and Neifakh, 1964; Smithberg, 1966).

The proteins of the zona radiata of the herring egg closely resemble ichthulin in their elemental and amino acid composition, so that it has even been suggested (with little foundation) that the membrane proteins are insoluble forms of ichthulin (Steudel and Osato, 1923a; Iguchi, 1942).

TABLE 14. Ratio between size of spermatozoon and micopyle diameter

Species	Width of spermatozoon head, μ	Diameter of terminal canal of micropyle, μ	Author
Clupea harengus membras	2		Kupffer, 1878
Clupea harengus harengus		2.5	Hoffmann, 1881
Clupea harengus pallasi	1.5	1.5	Yanagimachi, 1957b; Kanoh, 1957
Oncorhynchus keta	~3	~ 3	Kanoh, 1957
Salmo salar	3.5–4	3–4	His, 1873
Salmo trutta m. lacustris	3	3	Blanc, 1894
Carassius carassius	3.2	3.5–4	Kobayasi, 1963
Crenilabrus griseus	1.7–1.8	Slightly more than 2	Hoffmann, 1881

The properties and amino acid composition of zona radiata proteins of salmon eggs seem to belong neither to typical keratins, collagens nor mucoproteins; according to the proportions of histidine, lysine and arginine these proteins are closest to keratins and may qualify as pseudokeratins (Young and Inman, 1938). The effect of various solvents on the trout egg membrane indicates a similarity to the cuticle of invertebrates (Brown, 1955).

1.2.7. Comparison of Egg Structure in Different Groups of Fishes

The eggs of fishes belonging to different taxonomic groups are similar in having the same general type of structure (containing a large amount of unevenly distributed yolk inclusions) and a nucleus retarded at the same stage of meiosis. Apart from this, there are substantial differences among them.

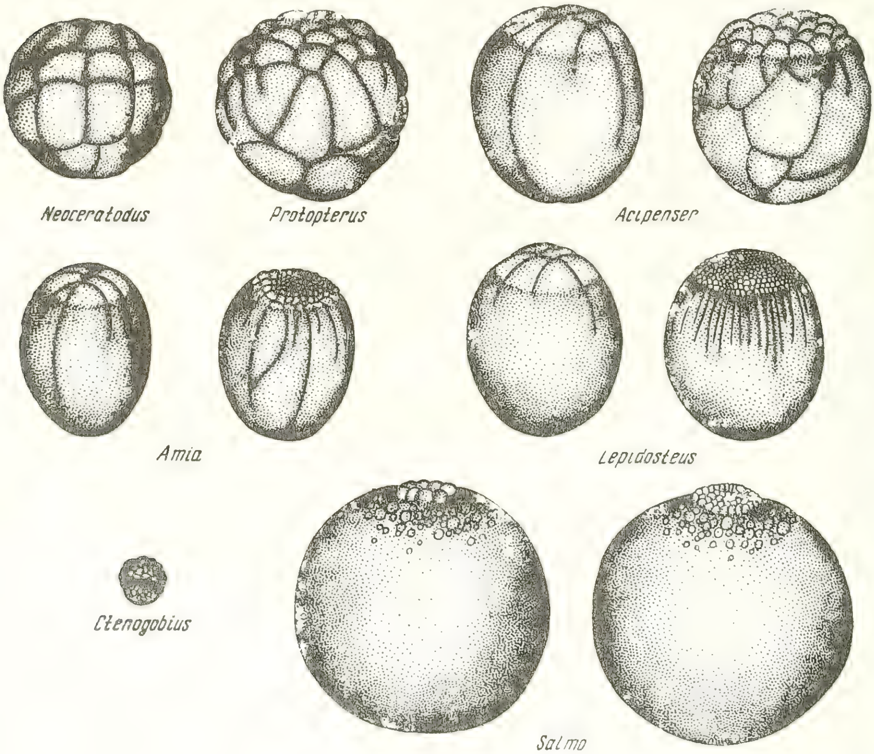

FIGURE 18. Different types of cleavage of fish eggs in relation to differing yolk concentrations (schematic):

Neoceratodus (after Semon, 1901); Protopterus (after Kerr, 1909); Acipenser (original, and after Ginzburg and Detlaf, 1955); Amia (after Whitman and Eycleshymer, 1897); Lepidosteus (after Eycleshymer, 1899); Ctenogobius (after Kuntz, 1916); Salmo (original).

The most striking differences are in morphology and concentration of yolk inclusions, which may be judged from egg cleavage. As the concentration of yolk inclusions increases, there is a consecutive transition series from typical holoblastic cleavage (complete and almost regular, found in eggs with less yolk than in fishes, e. g., sand lance eggs) to meroblastic cleavage in which only a small part of the egg divides. In lungfishes, chondrostean fishes and bowfin, cleavage is complete but irregular; in the series indicated, this irregularity increases progressively (Figure 18). Gar eggs, richer in yolk, constitute the next member of the series: their cleavage is already incomplete, but the furrows go beyond the limits of the animal pole, which corresponds to the blastodisc of meroblastic eggs. Selachian and teleost eggs are characterized by the highest concentration of yolk inclusions and may be regarded as typical meroblastic eggs; the abundance of yolk impedes division of the entire egg and the cleavage furrows do not extend beyond the limits of the blastodisc, which is rich in cytoplasm and clearly delimited from the rest of the egg (see Waldeyer, 1960; Shmidt, 1951, 1953; Nelsen, 1953). These differences in yolk concentration affect not only cleavage but also gastrulation, organogenesis, etc.

Not only the yolk concentration is important for embryo development, but also the absolute amount of yolk. The greater the yolk reserve, the longer the embryonic development within protective membranes or without exogenous food, and the more the active larva resembles the mature organism. In selachians and chimaeras, eggs usually have immense yolk reserves which allows the embryo to develop for many months, sometimes more than a year; at the end of this period the young resemble adults in all main structural features. Among teleosts, salmonid larvae emerging from yolk-rich eggs are much more differentiated and similar to adults than larvae from small pelagic eggs of marine fishes; accordingly, the larval period lasts much longer in the latter eggs.

In viviparous selachians and teleosts, the eggs often contain relatively little yolk. This deficiency is compensated by nutritive substances from the female, giving the same results as in eggs having large amounts of yolk. In extreme cases, differentiation in the uterus may be considerably extended. The yolk-deficient eggs of Embiotocidae hatch sexually mature individuals (at least, sexually mature males).

A particular concentration of protein and fatty yolk inclusions (as well as certain other relatively stable features of egg structure) is characteristic for related forms, and represents an important systematic character (Rass, 1936). The systematic importance of such characters may be illustrated among flatfishes. Eggs of the subfamilies Pleuronectinae and Hippoglossinae contain no separate fatty inclusions. Eggs of the subfamily Soleinae contain numerous oil droplets, whereas in the subfamily Bothinae there is a single large oil drop (see Cunningham, 1897).

These variations in concentration and amount of yolk inclusions affect morphogenesis in the embryo and the duration of the entire embryonic period, but they have no particular importance in the initial stage of development which interests us here, viz., fertilization.

In relation to this, an important role is played by structural features related to the cortical layer and egg membranes: the presence or absence of cortical bodies whose contents are discharged at fertilization and the accessibility of greater or lesser parts of the egg surface to the spermatozoons. The decisive importance of these features for the type of fertilization — physiological monospermy or polyspermy — will be examined in detail later.

1.3. CAVITY FLUID

Ovulated eggs are immersed in a more or less viscous protective fluid, in which eggs remain fertile longer than in any other medium. The origin of this fluid is unclear. In salmonids, it apparently appears only at the moment of ovulation (Greene, 1940), and may be discharged from the follicles destroyed at ovulation. In sturgeons, quite a large amount of fluid is present in the body cavity already several hours before ovulation (Detlaf, unpublished), and thus must be an exudate of the ovary or of the body wall.

In investigations of various fish species, different names have often been given to this fluid, e.g., coelomic (peritoneal) or ovarian fluid. This is related to differences in structure of the female reproductive system in the different fishes (Shmal'gauzen, 1947; Suvorov, 1948; Hoar, 1957; Gérard, 1958). In selachians, lungfishes, chondrosteans and in the bowfin, the eggs ovulate in the body cavity and emerge through Müller's canal, which serves as an oviduct. In the gar and most teleosts, the ovary is a hollow sac extending directly into the oviduct, which arises independently of Müller's canal. In some teleosts — representatives of Clupeiformes (from the Salmonidae, Osmeridae, Argentinidae, Osteoglossidae, Hyodontidae, Notopteridae, Galaxiidae), Anguilliformes (from Anguillidae and Murenidae) and also among Cypriniformes (in Misgurnus) — oviducts are degenerate and eggs enter the external milieu from body cavity through a funnel or genital pore (depending on degree of reduction of the oviducts). Depending on the structure of the reproductive system, the fluid in which the ovulated eggs are immersed is called coelomic or peritoneal in fishes such as sturgeons and salmons, ovarian in cyprinids, percids and many other fishes. Since the functions of this fluid are identical in all fishes and its origin is unclear, it seems superfluous to make such a distinction. It is best in all cases to call it cavity fluid (as do several authors).

The cavity fluid of fishes is colorless or slightly yellowish. In salmonids it is aqueous, transparent or slightly opalescent; in sturgeons it is usually viscous and rather turbid. The cavity fluid contains organic matter and inorganic salts. The fluid in salmonids has been best studied. Its dry residue is less than 2%: 1.58—1.73% in rainbow trout (Scheuring, 1925), 1.30% in salmon (Hayes et al., 1946). Correspondingly, its specific weight varies between 1.008 and 1.018 in rainbow trout and on the average 1.007 in salmon. The predominant cation in salmon is sodium, the predominant anion chlorine; the ratio between different ions corresponds to that of seawater and blood plasma.

Correlation of data on the freezing point depression (Δ, cf. Table 15) indicates that in salmonids the cavity fluid is somewhat hypotonic with respect to blood plasma and contents of the ovulated egg.

TABLE 15. Freezing point depression (Δ) of blood, cavity fluid and egg contents in salmonids

Species	Δ			Author
	blood	cavity fluid	eggs in body cavity	
Oncorhynchus tschawytscha	0.610	0.549		Green, 1904
Salmo salar		0.560		Atkins, 1911
		0.555*	0.599**	Hayes et al., 1946
	0.61			Parry, 1961
Salmo irideus	0.645			Green, 1904
	0.518	0.535		Scheuring, 1925
Salmo salvelinus	0.636		0.645	Runnström, 1920
Salvelinus (Salmo) fontinalis	0.615		0.640	Bogucki, 1930

* Calculated according to cation content.

** Calculated according to Na^+ and K^+ content.

In rainbow trout, the pH of the cavity fluid is 7.9—8.0 and that of the blood serum 7.6 (Scheuring, 1925).

The cavity fluid in perch is similar in composition to that of salmon, but it differs considerably in the threespine stickleback, containing 1.5 times the salt content and many times the calcium content (Thomopoulos, 1953a).

1.4. EGG FERTILITY AND ITS PRESERVATION UNDER DIFFERENT CONDITIONS

If egg maturation takes place under normal conditions, the ovulated eggs generally are highly fertile. For example, we observed that fertility of lake trout eggs kept in river ponds of the Svirskii fish breeding station, often reached nearly 100% when artificially fertilized. Eggs of Acipenser güldenstädti and A. stellatus obtained at the Rogozhkinskii sturgeon-breeding station by the method of hypophyseal injection in good-quality batches often reached 90—95% fertility or more. There are also data on the high fertility of naturally spawned eggs. For example, in nests of Pacific salmon with eggs spawned under normal conditions there were either no unfertilized eggs or no more than in the case of artificial fertilization (Kuznetsov, 1928).

After the ovulated egg is spawned in the water, its fertility gradually decreases (sometimes within a very short time) and finally is lost completely. The same occurs if the ovulated egg is kept in any other medium. Moreover, the rate of loss of fertility differs greatly in different fishes and depends largely on the medium and conditions under which the eggs are kept (see Table 16).

Table 16 shows the longest periods in which fertility is preserved in at least part of eggs. It is seen that if eggs of some fishes (lake trout, chinook salmon, cisco, pike, Diptychus, Schizothorax and others) are kept in water (with salinity corresponding to spawning conditions),

TABLE 16. Preservation of egg fertility in various media

Family	Species	Temperature, °C	Period of preservation of fertility: in water with salinity corresponding to spawning conditions	in physiological salt solution	in cavity fluid	Author
A. Freshwater spawners						
Acipenseridae	Huso huso	12–13.5			18 hr	Ginzburg, unpublished
	Acipenser güldenstädti colchicus	14.1–15.4	4–12 hr		12–18 hr	Ginzburg, unpublished
	Acipenser stellatus	23–25	Up to 10 hr			Persov, 1941
		19.4–21.2	6–8 hr	8–16 hr	8–16 hr	Ginzburg, unpublished
Clupeidae	Caspialosa kessleri pontica		> 2 hr			Kryzhanovskii, 1956
	Caspialosa volgensis	3.5			24 hr	Stroganov, 1936
		26			1.3 hr	
			7 min			Stroganov, 1938b
Salmonidae	Oncorhynchus keta		15–30 min			K. Yamamoto, 1951b
		0–5			> 72 hr	Hamano, 1957
	Oncorhynchus gorbuscha	9.8–10.1	15 min			Smirnov, 1963b
		8–9			105 hr	Withler and Humphreys, 1967
	Oncorhynchus nerka	2–8	28 min		96 hr	Foerster, 1965
		8–9			130 hr	Withler and Humphreys, 1967
	Oncorhynchus tschawytscha		5 min	>25 min	16 hr	Rutter, 1904
	Salmo salar	3.4	>12 min			Novikov, 1953
	Salmo trutta m. lacustris	1.7–8.9	1.5 min			Ginzburg, unpublished
		0.4–1.0		240 hr	360 hr	
		10–12.5		48 hr	72 hr	
	Salmo irideus	0–5			>72 hr	Hamano, 1957
	Salmo salvelinus		< 30 min	72 hr		Runnström, 1920
	Coregonus albula		4 min			Lindroth, 1947

Esocidae	Esox lucius	10	1 min			
		24	0.5 min			
		3.5			60–192 hr	Lindroth, 1946
		8			48–96 hr	
		13			24–48 hr	
Cyprinidae	Chalcalburnus chalcoides	18–22			>6 hr	Evtyukhin, 1933
	Vimba vimba vimba carinata	18–22			>6 hr	Evtyukhin, 1933
		16–18	45–60 sec		6–8 hr	Smirnova and Kuz'mina, 1966
	Carassius auratus		<1 min			Hamano, 1951
	Acheilognathus lanceolata	19–23	25–30 min			Gamo et al., 1960
Cobitidae	Misgurnus anguillicaudata	19–23	5 min			Gamo et al., 1960
Cyprinodontidae	Oryzias latipes		4 min	6 hr		T. Yamamoto, 1944a
					2 hr	Sasaki and Ito, 1962
Percidae	Lucioperca lucioperca	4–10			>8 hr	Evtyukhin, 1933
B. Brackish-water spawners						
Clupeidae	Clupea harengus membras		4 hr			Kryzhanovskii, 1956
Cyprinidae	Schizothorax pseudaksaiensis issykkuli		<3 min			Turdakov, 1962
	Diptychus dybowskii		<3 min			Turdakov, 1962
C. Salt-water spawners						
Clupeidae	Clupea harengus harengus	4	120 hr		192 hr	Blaxter, 1955
Cyprinodontidae	Fundulus heteroclitus		60–75 min			Newman, 1905
			10–30 min			Kagan, 1935
Ammoditidae	Ammodytes tobianus		6 min			Thomopoulos, 1954
		5			12 hr	
Cottidae	Cottus bubalis euphrasen	13	>10 min			Thomopoulos and Bauchot, 1957
		5–6			48 hr	
Pleuronectidae	Limanda schrenki	6–8	<2 hr	5 hr		K. Yamamoto, 1951a
Gasterosteidae	Gasterosteus aculeatus		<3 hr			Thomopoulos, 1953b

fertility is preserved for no more than several minutes. Such a rapid loss of fertility is related to the fact that these eggs become activated during the first few minutes in the water, making sperm penetration impossible. In many other fishes, activation occurs later or else not at all, and the eggs remain fertile for a considerable period; in some of them, e.g., in Acipenser güldenstädti, A. stellatus and Atlantic herring, some of the eggs may be fertilized even after many hours in water.

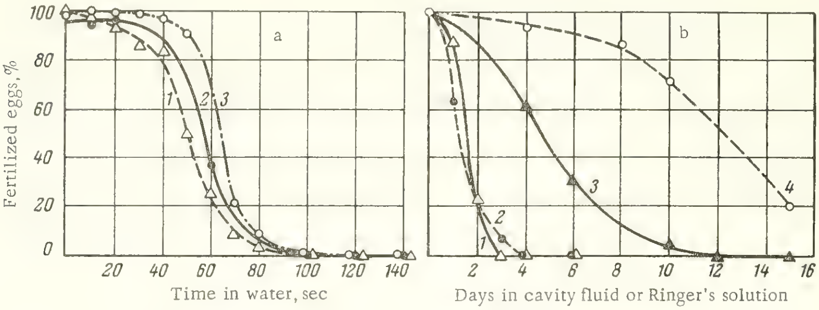

FIGURE 19. Preservation of fertility in lake trout eggs in water, Ringer's solution and cavity fluid: a — eggs of 3 females kept in water; b — eggs of one female kept in Ringer's solution (1, 3) and in cavity fluid (2, 4). Incubation temperatures: 1 and 2 — 10.0–12.5°C; 3 and 4 — 0.4–1.0°C.

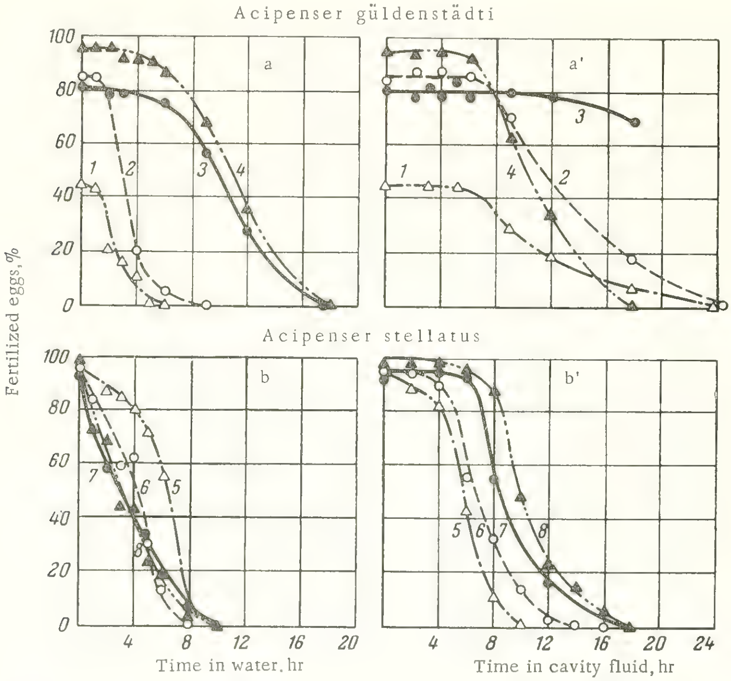

FIGURE 20. Preservation of fertility in eggs of Acipenser güldenstädti and A. stellatus in water (a, b) and in cavity fluid (a', b'). (Number of curve indicates the particular female).

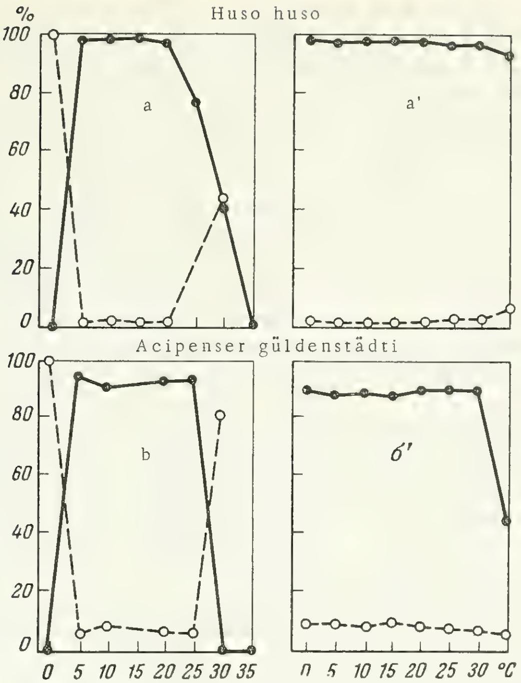

FIGURE 21. Effect of temperature on fertility of gametes of sturgeon:

a and b — fertility of eggs; a' and b' — fertility of spermatozoons. In each experiment (on Huso huso and Acipenser güldenstädti) gametes from a single pair of spawners were used. The continuous line denotes fertilized eggs and the broken line activated eggs.

Eggs remain fertile much longer in a physiological salt solution than in water. The eggs of Oryzias latipes retain this capacity for 4 min in water and for 6 hr in Ringer's solution; the eggs of lake trout retain fertility for only 1.5 min in water, but for 48—240 hr (depending on the temperature) in Ringer's solution.

Eggs retain fertility longest in cavity fluid. When temperature is lowered the duration of fertility is increased several times: in pike from 1 to 2 days at 13°C to 8 days at 3.5°C, in lake trout from 3 days at 10—12.5°C to 15 days at 0.4—1.0°C (Table 16).

The eggs in a particular batch do not become sterile simultaneously, so that the percent reduction in fertility begins considerably earlier than indicated in Table 16. Thus, let us examine the kinetics of the loss of fertility in lake trout, Acipenser güldenstädti and A. stellatus (Ginzburg, unpublished).

When placed in water, all the lake trout eggs remain fertile for 40—50 sec. but then rapidly lose their fertility so that all are sterile after 100—120 sec (Figure 19, a). In cavity fluid and Ringer's solution the reduction in fertility is comparatively slower and lasts many hours (Figure 19, b). The loss of fertility is particularly slow at low temperatures (0.4—1.0°C); thus, 85.3%

of eggs kept in cavity fluid at low temperatures for 8 days remain fertile and 19.3% after 15 days. Loss of fertility is more rapid in Ringer's solution than in cavity fluid. Eggs fertilized after 45 days in cavity fluid had normal first cleavage divisions, whereas eggs fertilized after 8 days in Ringer's solution showed a disrupted cleavage pattern; subsequent development of these eggs was not followed.

Sturgeon eggs are considerably more resistant to the effect of water than those of lake trout and other salmonids. Reduction in egg fertility of different individuals proceeds at different rates. In Acipenser güldenstädti colchicus the percent fertility in two batches of eggs was sharply reduced during the second hour (Figure 20, a, 1, 2), while in two other batches (3, 4) after 6 hours in water. Sterility appeared earlier in A. stellatus (during the first hour the eggs were kept in water) than in A. güldenstädti (Figure 20, b).

The fertility of A. güldenstädti and A. stellatus eggs remained at a high level in cavity fluid, as a rule much longer than in water (Figure 20, a', b'). However, there are also exceptions: in eggs of 2 females out of 8 studied (numbers 4 and 5), the kinetics of fertility loss was parallel in water and cavity fluid. This may be connected with the composition of the cavity fluid in sturgeons, which varies greatly in viscosity and probably also in concentrations of various constituents.

There are almost no data on the effect of temperature on fertility in sturgeon eggs. Only results of experiments in which eggs (or spermatozoons) of sturgeons were kept for a short time at various temperatures in the 0–35°C range are at our disposal (Ginzburg, unpublished). Portions of the eggs in cavity fluid (or "dry" spermatozoons) were subjected to the various temperatures for 60–100 min, after which insemination was carried out using gametes of the opposite sex which were kept at a constant temperature close to that at which the brood stock matured. Six such experiments on eggs of Huso huso, Acipenser güldenstädti and A. stellatus gave uniform results: eggs kept at temperatures from 5° to 20–25°C showed no substantial changes in fertility (Figure 21, a, b), whereas incubation at 0° or 30°C caused activation of the eggs and led to a sharp reduction in their fertility, usually to zero; irreversible damage was observed at 35°C.

It is clear that fertility of sturgeon eggs is preserved in the 5–20°C range; however, elucidation of optimal temperatures requires further investigation.

Chapter 2

THE SPERMATOZOON

The structural features of spermatozoa of various fishes and their distribution in the sperm are determined largely by the method of insemination.

In fishes which have retained the original method of insemination by emission of gametes into the water (namely, lungfishes, ganoids and most teleosts), male gametes may be considered as primitive type flagellate spermatozoa (see p. 115). Such spermatozoa are always distributed individually in the seminal fluid.

In fishes which have evolved internal insemination (selachians, chimaeras, some teleosts), the spermatozoon approaches the egg in the female's genital ducts in conditions very different from the aquatic medium; accordingly, the structure of the spermatozoa has changed. In addition, a special adaptation for internal insemination to reduce losses of spermatozoa involves agglutination of spermatozoa in large aggregates, spermatophores and spermozeugmas.

This description of fish spermatozoa includes considerable information on the structure of its main parts: head, middle piece and tail. The same section will deal briefly with the origin of the various organelles of the spermatozoon and their transformation during spermatogenesis. Afterwards, the structure of spermatozoa in various groups of fishes will be examined along with data on the distribution of spermatozoa in the ejaculate in fishes with external and internal fertilization and on properties of the spermatozoa (motility in various media and their fertilizing capacity).

2.1. STRUCTURE OF THE SPERMATOZOON

2.1.1. Head

The shape of the sperm head varies in different fishes, but is stable in each species. In some fishes it is almost spherical, either regularly spherical, ovate or acorn-shaped (most teleosts); in other fishes there are rod-shaped (sturgeons and some teleosts), spear-shaped (lungfishes), and elongate-cylindrical sperm heads (selachians, chimaeras and crossopterygian fishes), see Figures 23—25, 27—30, Plate V.

The greater part of the head is occupied by the nucleus, adjacent to which there is an acrosome in many fishes.

Structure of the nucleoplasm. The nucleoplasm of the sperm head shows an intense Feulgen reaction and strong basophilia, indicating high DNA content. Owing to the high density of the nucleoplasm, it appears structureless and homogenous in light microscope sections.

An idea of the structure of the spermatozoon nucleoplasm may be obtained in ultramicroscopic studies of its formation during spermatogenesis. Unfortunately, there are for fishes only a very brief description of the spermatid structure in Fundulus (Ris, 1959) and a work on spermatogenesis in the guppy (Porte and Follenius, 1960); in the latter work no attention was paid to the reorganization of the spermatid nucleus. Therefore, we must rely on descriptions of spermatogenesis in other animals which have common features of structure and reorganization of the nucleoplasm (Ris, 1959, 1961).

In primary spermatocytes of various animals, the nucleoplasm has a structure characteristic of interphase nuclei of somatic cells; it consists of a ball-shaped mass of coiled filaments about 100 Å thick, called "primary chromonemata" which are the basic elements of the chromosomes of all living animals cells. The primary chromonemata are often coiled in coarser fibers about 200 Å thick. Later, when the histone in the deoxyribonucleoprotein is converted to protamine in the spermatid nucleus (see p. 92), the nucleoplasm seems to consist not of these primary chromonemata, but of still finer filaments about 40 Å in diameter, which apparently are macromolecules of nucleoprotamine, which have a diameter of 30 Å in their native state (Zubay and Doty, 1959). Such a transformation in the nucleoplasm structure is observed in a number of animals (cephalopods, some insects, etc.), whereas in the sea urchin filaments 100 Å thick are retained in the mature spermatozoon (Afzelius, 1955). Moreover, during spermatogenesis in sea urchin there is no conversion of histone into protamine (Hamer, 1955). The regular change in ultrastructure with chemical reorganization of deoxyribonucleoprotein in the spermatid nucleus and the maintenance of previous ultrastructure in the absence of such reorganization indicate a causal relation between the appearance of the fine filaments of 40 Å diameter and the replacement of histone by protamine.

The mechanism of reorganization of the spermatid nucleus ultrastructure is not yet clear. According to the well-substantiated hypothesis of Ris (1959, 1961, 1966), each primary chromonemata consists of two nucleoprotein macromolecules joined by labile histone bridges. On replacement of the histone by protamine, the macromolecules break apart and become discernible with the electron microscope.

During spermatogenesis, the fibrils unite progressively into larger structures, both before and after the primary chromonemata are replaced by the fine filaments of 40 Å diameter (Ris, 1959, 1961).

The union of the microfibrils proceeds differently in different animals. In invertebrates with elongated and filiform sperm heads (gastropod and cephalopod mollusks, orthopteran insects), the microfibrils first uncoil and orient along the long axis of the nucleus, then they unite into still larger bundles or membranes. The spaces between these structures gradually diminish and finally disappear. In most vertebrates studied, e.g., in Fundulus (Ris, 1959), toad (Burgos and Fawcett, 1956), cat (Burgos and Fawcett, 1955) and man (Fawcett and Burgos, 1956; E. Horstmann, 1961), the fibrils remain coiled and dispersed randomly throughout spermatogenesis);

in sections of the spermatid nucleus, the fibrils rolled into a ball appear granular. Later the "granules" form large dense concentrations. The interstices between the concentrations of microfibrils then disappear, and a single dense nuclear mass is formed.

The orderly arrangement of the deoxyribonucleoprotein macromolecules in the elongated sperm heads is also undoubtedly preserved until the end of spermatogenesis due to the dense packing of these macromolecules whose individual structural elements can no longer be observed with the electron microscope. This is attested to by the intense specific (internal) double light refraction of the head piece of mature spermatozoa, negative in relation to the long axis of the head (Schmidt, 1928, 1937; Pattri, 1932).

Double light refraction appears at a particular stage of spermatogenesis. The circular nucleus of the early spermatid is always isotropic. During elongation of the spermatid nucleus, anisotropy appears and increases, reaching its maximum in the mature spermatozoon (Pattri, 1932; Hilden and Sato, 1963). The increased anisotropy is parallel to orientation of the nucleoprotein filaments (Kaye, 1958b; Hilden and Sato, 1963).

There is a definite connection between the shape of the mature sperm head and the intensity of anisotropy of its nucleoplasm. Spermatozoa with long, narrow heads — filiform, elongate-cylindrical or coiled (e.g., those of cephalopods and orthopteran insects) display strong anisotropy. Spermatozoa of the guppy and swordtail with relatively short rod-shaped heads display weak anisotropy, scarcely discernible in single spermatozoa. When many spermatozoa of these fishes are arranged in parallel or in several layers, anisotropy appears more distinctly. It becomes clearly discernible even in individual spermatozoa dehydrated with alcohol. The spherical sperm heads of the polychaete Arenicola, the mollusk Mactra and the threespine stickleback Gasterosteus aculeatus are isotropic.

Anisotropic sperm heads, like aggregates of orientated crystals, display dichroism (pleochroism) in polarized light when stained with various organic dyes and treated with gold chloride (Schmidt, 1928; Pattri, 1932), as well as in ultraviolet light (Casperson, 1940; Wilkins, 1951). The sperm heads of orthopteran insects are characterized by a high coefficient of dichroism (3:1 or more at a wavelength of 2650 Å). This indicates that a very high percentage of the nucleoprotein molecules is orientated along the long axis of the head. Such a degree of organization is not peculiar to a biologically active substrate; in this respect, sperm heads resemble the nucleoprotein crystals of plant viruses (Wilkins, 1951).

The results of X-ray diffraction analysis of spermatozoa (Wilkins and Randall, 1953; Wilkins, 1956) conform with data on their ultrastructure and optical properties. The sperm heads give an X-ray diffraction pattern corresponding to that of aggregates of microcrystals. In the case of cephalopods (cuttlefish and squid), the resulting patterns correspond to aggregates in which the microcrystals are orientated with their axes almost (but not quite) parallel. In the case of trouts (Salmo trutta) and char (Salvelinus fontinalus) the microcrystals are arranged more randomly. This difference is evidently determined by the spatial distribution of the nucleoprotein macromolecules.

In the case of fishes the following conclusion may be reached. Where the sperm head is spherical or acorn-shaped, as in most teleosts, the nucleoprotein macromolecules remain helical and randomly arranged. This is corroborated by ultramicroscopic investigations of the spermatids of Fundulus (Ris, 1959), by study of the double light refraction of stickleback spermatozoa (Pattri, 1932) and of X-ray diffraction in the sperm heads of trout and char (Wilkins and Randall, 1953). In rod-shaped sperm heads of platys there may be some orderly spatial organization of deoxyribonucleoprotein macromolecules since, unlike those of the stickleback, these spermatozoa display weak anisotropy (Pattri, 1932). There are no data on the spatial distribution of deoxyribonucleoprotein filaments in the elongated coiled sperm heads of selachians and chimaeras, but analogous to findings on spermatozoa with elongated heads in other animals it may be assumed that the macromolecule filaments uncoil and orient regularly along the long axis of the sperm head.

TABLE 17. Chemical composition of sperm nucleoplasm (in %)

Species	DNA	Protamine	Nonbasic proteins	Author
Acipenser güldenstädti colchicus	42.2	45.7	12.1	Georgiev, Ermolaeva and Zbarskii, 1960; Ermolaeva, 1964
Cyprinus carpio	38.1	46.9	15.0	After Vendrely et al., 1960
Esox lucius	39.0	40.8	20.2	
Salmo irideus	48.0	33.0	19.0	
Salmo salar	48.4	48.8	2.8	Zbarskii and Ermolaeva, 1961; Ermolaeva, 1964

The nucleus of the head piece is covered by a distinctive double membrane.

Chemical composition of the nucleoplasm. The nucleoplasm of the sperm head consists mainly of deoxyribonucleoprotein (DNP). Thus, in sturgeon spermatozoa DNP constitutes 90.5% and in salmon an average of 98.1% of the total nucleoplasm (Georgiev, Ermolaeva and Zbarskii, 1960; Zbarskii and Ermolaeva, 1961; Ermolaeva, 1964). The DNP of the spermatozoa of most fishes consists of nucleoprotamines: the neutral salt of deoxyribonucleic acid with basic protamine. In this respect, fish spermatozoa are similar to mollusks and certain animals; in the rest, the DNA of the sperm head, as in somatic cells, is associated with histones.

The DNA content in sperm heads is very high, constituting 38–48% of the nucleoplasm (Table 17); nuclei of most somatic cells contain 15–30% DNA (Mirsky and Ris, 1949; Allfrey et al., 1952; Georgiev, Ermolaeva and Zbarskii, 1960).

The DNA content in sperm heads is constant for each species and equal to the DNA in the haploid set of chromosomes; this equals half the DNA in nuclei of diploid somatic cells of the same species (Boivin et al., 1948; Vendrely and Vendrely, 1948; Mirsky and Ris, 1949; C. Vendrely, 1952; R. Vendrely et al., 1960; Sandritter et al., 1960); see Table 18.

The absolute amount of DNA per spermatozoon is almost the same in representatives of a single family, but differs considerably in different groups of fishes. This may be seen from the variation in DNA content in the erythrocyte nuclei of various fishes (Mirsky and Ris, 1951a), which is twice the amount of DNA in the sperm heads. The amount of DNA in the nucleus of a single erythrocyte (expressed in 10^{-9} mg) in sharks is 5.46–6.67, in lungfishes (Protopterus) 100, in chondrostean fishes (Acipenser sturio) 3.2, in bowfish (Amia) 2.3 and in teleosts (determination for 31 species) from 0.94 to 3.49. Furthermore, in all 3 representatives studied in the Lutianidae it was 2.1, in the Scaridae it varies between 2.45 and 2.58 and in the Carangidae between 1.21 and 1.35.

TABLE 18. DNA content (in 10^{-9} mg per nucleus) in somatic cells and spermatozoa (after Mirsky and Ris, 1949)

Species	Erythrocyte	Hepatic cell	Spermatozoon	Double spermatozoon content
Herring	1.97	2.01	0.91	1.82
Carp	3.49	3.33	1.64	3.3
Sea trout	5.79		2.67	5.34
Toad	7.33		3.70	7.40
Gurnard	2.34	2.39	1.26	2.52

DNA extracted from the sperm heads of sturgeon had a molecular weight of 5.2×10^6 (Shmerling, 1965a). This is close to the molecular weight in purified preparations from various tissues of animals and from bacteria ($6-8 \times 10^6$). The in vivo molecular weight of DNA is apparently considerably higher. In particular, the results of detergent extraction of DNA from trout sperm heads (Davison, 1960) results in preparations with an unusually high coefficient of sedimentation (distribution of $S_{20, \omega}$ between 10 and 90 with maximum at 45 S) and a higher viscosity than in preparations obtained by the usual methods.

Protamines, the second DNP component in the spermatozoa of most fishes, are simple proteins (see Kossel, 1929; Felix, 1960; Busch, 1965) having a low molecular weight of $4-12 \times 10^3$. The fish protamines contain a small number (usually 6–8) of various amino acids, predominantly basic amino acids, mainly arginine; arginine nitrite (as percent of total protamine nitrite) is 67.4% in sturine (the protamine of Acipenser sturio), 84.5–91.7% in salmine (the protamine of Salmo salar and Oncorhynchus keta) and 86.9–91.2% in clupeine (the protamine of Clupea harengus). Accordingly, protamines have clearly expressed basic properties. Apart from arginine, all the protamines studied contain alanine and serine, and many contain proline, valine, glycine and isoleucine. Protamines from spermatozoa of some fishes also contain histidine, lysine, threonine and glutamic acid. The protamines studied do not contain tryptophan, phenylalanine or sulfur-containing amino acids.

The amino acid composition in the protamines varies in different kinds of fishes. Some differences were found even between different forms within a single species. Thus, glycine is a constant component in clupeine of Clupea harengus pallasi (Ando et al., 1959), whereas glycine was found only in rare cases in the clupeine of C. harengus harengus (Felix, 1960). On the other hand, in two interbreeding species of Salmonidae – Salmo trutta and Salmo (Salvelinus) fontinalis – the protamine is of uniform composition (Felix et al., 1951). It may be assumed that differences in protamine composition are an obstacle to interspecific crossbreeding.

As already indicated, protamine appears in the gametes at the end of spermatogenesis, whereas in earlier stages the deoxyribonucleoprotein of the male gametes, like in somatic cells, consists of histone. As in the case of salmonids, the conversion of histone into protamine is accomplished rapidly during transformation of the spermatids (Miescher, 1874, 1896; Alfert, 1956; Felix et al., 1958; Felix, 1960). In some fishes, as also in many other animals, histone does not change into protamine. Thus, histones were extracted from spermatozoa of cod, burbot and Centrophorus granulosus (Kossel, 1929); histochemical investigations indicate that histones are also present in mature spermatozoa of carp and roach (Butskaya, 1965).

Where histones are preserved until the end of spermatogenesis, a change is observed in their composition. Corresponding data for fishes are not available. In the case of two dragonfly species, the histone of the spermatozoan nucleus becomes enriched with arginine during spermatogenesis (Seshachar and Bagga, 1963); the same has been established for mammals. In the last stages of spermatogenesis in the mouse, intensive incorporation of ^{3}H-arginine is observed, whereas other amino acids are not incorporated or else weakly; at the same time the staining by histochemical methods of the nuclear histone is changed. It is evident that new, arginine-rich nuclear histone is synthesized in the spermatids (Monesi, 1965). In the bull, the basic properties of the nuclear proteins are sharply strengthened and their arginine content increases (Sakagusha reaction) as the spermatid becomes a mature spermatozoon (Gledhill et al., 1966).

The biological significance of protamine replacement of histones is apparently connected with the high arginine content of the former.

It is known that positively charged histones readily unite with negatively charged polymers, primarily with DNA; they form complexes with many enzymes, their substrates and cofactors. Such properties of the histones have wide inhibitory effects on biosynthetic processes; in particular, they suppress matrix activity of DNA (see Busch, 1965; Ganelina, 1965).

Experiments indicate that arginine-rich histones are much stronger inhibitors than total histone preparations (Allfrey et al., 1963). It may be that protamines, usually containing more than 80% arginine, are particularly strong inhibitors and ensure discontinuation of biosynthetic processes in the nucleus during the prolonged period from formation of the spermatozoa, until their ejection from the testes and union with eggs. In many fishes with spring-summer spawning this period lasts about half a year or more. Spermatozoa are already fully formed in the fall and males overwinter in a mature state, whereas spawning takes place only in April-June. This is observed, for example, in sterlet (Ol'shvang, 1936), stellate sturgeon and sturgeon (Persov, 1947a, b), perch (Turner, 1919; Kulaev, 1927), catfish (Kulaev, 1944), pike perch, carp (Butskaya, 1955) and pike (Zaitsev, 1955).

The significance of chemical reorganization of the nucleus for the synthetic processes in spermatids is indicated by data obtained in locusts (Schistocerca gregaria). While young spermatids actively synthesize RNA, synthesis of RNA is suddently discontinued when the nucleoplasm begins to condense, which coincides with the process of conversion of typical histones into arginine-rich proteins (Das et al., 1965).

After fertilization, when the new organism begins to develop and hereditary material must be activated, the nucleoproteins of the sperm nucleus change from protamines into histones. This process was traced in developing eggs of Helix aspersa (Bloch and Hew, 1960). The protamines of the pronucleus are replaced by arginine-rich histones; only at the end of cleavage do the nucleohistones of the embryo cells become indistinguishable from those of somatic cells of the mature organism.

In addition to nucleoprotamines, there are also proteins of a nonprotamine (nonbasic) type in sperm heads (Stedman and Stedman, 1947; Felix, 1960; Georgiev, Ermolaeva and Zbarskii, 1960; Zbarskii and Ermolaeva, 1961; Ermolaeva, 1964). The nonbasic proteins in spermatozoan nucleoplasm constitute from 2.8 to 20.2% (Table 17), whereas in somatic cells the content of such proteins is much higher, e.g., 44.8% in nuclei of hepatic cells, 40.9% in spleen cells 47.7% in brain cells of the rat (Georgiev, Ermolaev and Zbarskii, 1960). This difference confirms the relation between intensive cell metabolism and the content of nonbasic nucleoproteins (Mirsky and Ris, 1949; Mirsky and Osawa, 1961), and may be connected with the established differences in the organization of nuclei of spermatozoa and of somatic cells. The somatic cells have nucleoli containing acidic protein, whereas there is no nucleolus in the sperm head.

The nonhistone proteins present in the sperm head apparently participate (as in the nuclei of somatic cells) in formation of the chromosomes. The importance of these proteins in the continuity of chromosomal form is indicated by the following data. After extraction of histone from nuclei of mammalian liver, kidney and pancreas cells, the chromosomes maintained their shape; on the other hand, enzymatic digestion of DNA or the nonbasic proteins caused the chromosomes to lose their shape (Mirsky and Ris, 1951b). It seems likely that the nucleoprotein macromolecules in the chromosome are cross-linked by nonhistone proteins (Ris, 1966).

The structural elements of nonhistone protein of the chromosomes were studied ultramicroscopically in nuclei of somatic cells extracted in salt solutions (Georgiev and Chentsov, 1960, 1963). They were shown to consist of microfibrils 80–100 Å in diameter, bearing granules about 150 Å in diameter (ribosomes or precursors of ribosomes). These microfibrils, called nucleonemas by Georgiev and Chentsov, are identical with the structural elements of the nucleolus.

The sperm heads of fishes (cods, herrings, salmons and sturgeons) contain, in addition to the components mentioned, a small quantity of RNA (Mauritzen et al., 1952; Georgiev, Man'teva and Zbarskii, 1960; Zbarskii and Ermolaeva, 1961; Ermolaeva, 1964). RNA constitutes 2.5–5.3% of the acid protein fraction, or only hundredths of one percent of the entire nucleoplasm of the sperm head.

The low RNA content in spermatozoa is competant with the very low level or even absence of protein synthesis. There are no corresponding data available for fishes, but mature spermatozoa of the frog, rat and bull fail to incorporate radioactive amino acids (Martin and Brachet, 1959).

Finally, sperm heads of fishes yielded a small (by volume) globulin fraction (Georgiev, Ermolaeva and Zbarskii, 1960; Zbarskii and Ermolaeva, 1961). The globulin fraction of rat hepatic cell nuclei were shown to contain proteins and RNA of the nuclear sap (Zbarskii and Georgiev, 1959). The presence of such a fraction in sperm heads suggests that the spermatozoan nucleus does not consist merely of a number of chromosomes, but contains a small quantity of nuclear sap indiscernible under a microscope owing to the compact structure of the nucleus (Stedman and Stedman, 1947).

Reorganization of the structure and composition of the nucleoplasm during spermatogenesis is accompanied by profound changes in its metabolism. Inhibition of biosynthetic processes has already been mentioned. In addition, osmotic properties have been observed to change, and the spermatozoon develops an exceptional resistance to normally injurious conditions. Thus it was found for Loligo pealii (Hilden and Sato, 1963) that the maturing spermatozoon shows diminishing sensitivity to osmotic changes in the medium, and the mature sperm head does not change volume even in distilled water. Experiments by these authors with heavy water indicate that the spermatozoon of squids is water-permeable at all stages of development. Thus, the exceptional osmotic resistance of the mature spermatozoon cannot be explained by a change in membrane permeability, but probably results from the reorganization of the nucleoplasm.

Acetylcholine esterase activity was found in sperm heads of trout and perch (see Tibbs, 1962), which accords with the presence of acetylcholine and acetylcholine esterase in flagellate protozoa and infusoria and the concept of the role of acetylcholine as a regulator of autonomous rhythmic activity, in particular the rhythmic activity of cilia (see Burn, 1956; Buznikov, 1957).

Acrosome. The acrosome (earlier called the apical body and the perforator) is a constant component of flagellate spermatozoa (Wilson, 1925; Nath, 1956). Only rarely are spermatozoa devoid of a morphologically distinct acrosome, for example, in the hydra and ascidian (Franzén, 1956) or in teleosts (see below). Investigation of spermatogenesis of various invertebrates and vertebrates indicates that the acrosome is derived from the Golgi bodies, either by secretion or by direction transformation of one or several Golgi bodies (Bowen, 1922b; Nath, 1956; Beams et al., 1956; E. Horstmann, 1961).

In spermatocytes, the idiosome (cytoplasmic sphere around the centriole) is surrounded by Golgi bodies and forms with them a single complex. Prior to maturation divisions, this complex splits into numerous units (dictyosomes) which are distributed among the daughter cells. In the spermatid, the dictyosomes fuse into a single mass, the acroblast, which gives rise to the acrosome. The acrosome usually develops from a vacuole containing acrosomal granules, which separates from the acroblast, migrates to and comes in contact with the spermatid nucleus. Subsequently the acrosome becomes differentiated and assumes a definitive shape.

It was shown in a number of works (Grassé and Tuzet, 1932; Ratnavathy, 1941; Vasisht, 1953, 1954a) that the Golgi bodies in sharks and skates participate in formation of the acrosome. However, the details of this process are not consistent. According to Ratnavathy, the selachian acrosome, as in amphibians and mammals, appears as a vacuole containing an acrosomal granule; according to Vasisht, however, no acrosomal vacuole is formed and the compact proacrosomal granule is formed directly from one or several Golgi bodies.

In mature spermatozoa of all fishes except the teleosts, the acrosome is located in front of the nucleus and is shaped either like a thin conical outgrowth (selachians, chimaeras, lungfishes, crossopterygian fishes; see Figures 24 and 26) or a flat cap (sturgeons; see Figure 27). Teleost spermatozoa lack the acrosome.

There are no data on the chemical composition of the acrosome of fish spermatozoa. In mammals, the acrosome and its precursors give a positive PAS (periodic acid — Schiff) reaction, according to McManus and Hotchkiss. The precursors are rich in PAS-positive substances. As spermatozoa mature, the intensity of the reaction decreases, but, PAS-positive substances do not usually disappear completely (Leblond and Clermont, 1952; Clermont and Leblond, 1955; Surikova, 1957; Bishop and Walon, 1960). In Natrix sipedon confluens the acrosome contains only a very small amount of PAS-positive material at its tip (Schrader and Leuchtenberger, 1951). Much PAS-positive material is found in the acrosome of Glossiphonia complanata (Damas, 1965), Helix aspersa (Bradbury and Meek, 1963) and in Hemiptera (Schrader and Leuchtenberger, 1951; Moriber, 1956).

The PAS reaction is specific for carbohydrates containing the 1, 2-glucose group (Pearse, 1960). Histochemical study has shown that the carbohydrates in the acrosome are neither identical with glycogen or hyaluronic acid; in leeches and snails they are neutral polysaccharides.

The insect acrosome also contains proteins, and that of snails lipids.

The acrosome plays an important role in the gametic association, but its function is not mechanical as suggested earlier (hence, the name "perforator"). The acrosome contains lysins, which dissolve the egg membrane at the point of attachment of the spermatozoon, and it is the first part of the spermatozoon coming in contact with the ooplasm. The shape and structure of the acrosome then change radically, as will be considered in the next chapter.

The nuclear part of the head, which has its own membrane, and the acrosome are covered by a common plasmalemma, which extends uninterruptedly to the middle piece and tail. In the mature spermatozoon, it closely joins the acrosome and nucleus, and because of its extreme thinness is indiscernible under a light microscope. However, its presence in fish spermatozoa was detected using standard cytological methods during a study of spermatogenesis in selachians (Swaen and Masquelin, 1883), as well as in mature spermatozoa of various fishes upon maceration and plasmolysis of the head (Retzius, 1909; Kol'tsov, 1909).

2.1.2. Middle Piece

The middle piece, adjoining the sperm head, is formed by the centrosome and mitochondria of the spermatid (Wilson, 1925; Nath, 1956; Sotelo and Trujillo-Cenóz, 1958; Fawcett, 1961).

Investigations with the light microscope only elucidated the general outlines of the complex transformations of the centrioles of the centrosome of the spermatid during spermatogenesis. It was shown that the proximal centriole, lying close to the nucleus, comes into contact with the nucleus. The distal centriole, lying close to the periphery of the spermatid, gives rise to the axial filament of the tail, serving as its basal granule (see Figure 24, a). For some animals, including selachians and mammals, the division of the distal centriole into the basal granule of the flagellum and the "ring centriole" has been described.

Due to their minuteness, it was impossible to determine the shape and structure of the centrioles until recent electron microscope investigations, which have made it necessary to reexamine the previous terminology. The study of spermatogenesis in various animals has shown that the centrioles in spermatids, as in somatic cells, are cylindrical bodies. The centriole wall is formed by 9 groups of thin tubules parallel to the axis of the cylinder and enclosed in a dense matrix. The distal centriole retains its specific structure throughout spermatogenesis. From one end arise the axial filaments of the flagellum (which are firmly and uninterruptedly anchored in the distal centriole), from the other end fibrils unite it with the proximal centriole. The "ring centriole" of classical cytology in no way resembles a centriole, and its origin is unclear in many cases. It has been suggested that this formation be called the distal ring (Sotelo and Trujillo-Cenóz, 1958). During spermatogenesis the proximal centriole undergoes profound transformations and loses its original structure in many animals, sometimes disappearing completely. The above authors propose calling the transformed proximal centriole, the juxtanuclear body.

Derivatives of the centrioles are surrounded by a mitochondrial sheath; in selachians and chimaeras the sheath is highly developed and helical, but in most teleosts it has simpler structure. The physiological significance of mitochondria in the middle piece becomes clear if one remembers that mitochondria play a key role in providing the cells with energy, and contain a complete set of enzymes, cofactors and ancilliary substances necessary for oxidative phosphorylation; usually mitochondria are present in sections of cell requiring ATP (Novikoff, 1961; Lehninger, 1964). In the spermatozoon, the modified mitochondrial sheath surrounds the motile center of the spermatozoon; this apparently ensures the concentration of the energy-generating systems near structures requiring energy. Despite considerable differences in structure, the mitochondria of the middle piece biochemically resemble mitochondria of other cells (Mohri et al., 1965).

Data on the morphology of the middle piece of fish spermatozoa are scant and have been obtained mainly with the light microscope. Considerable differences in the middle-piece structure of spermatozoa have been indicated and will be discussed in the sections dealing with spermatozoa of various groups of fishes.

2.1.3. Tail

The tail, following the middle piece, is formed by an axial filament surrounded by a cytoplasmic layer and covered externally by the plasmalemma. As mentioned previously, the axial filament of the tail arises from the distal centriole.

In many animals (including some fishes) the sperm tail is differentiated into a long principal piece and a short end piece, at whose transition the tail diameter decreases sharply (see Figures 27, 30, c, 32; Plate V, c). Until recently it was thought that the plasmalemma terminates at this point, and that the axial fibrils are exposed in the end piece (Wilson, 1925). However, ultramicroscopic investigations of spermatozoa of sea urchin (Afzelius, 1959), salmonids (Lowman, 1953) and man (Ånberg, 1957) showed that the plasmalemma reaches the very end of the tail and that the change in tail diameter is related to the termination of some of the fibrils.

In some fishes the cytoplasm of the axial filament has considerable width.

The earliest cytological investigations of macerated fish sperm tails (Ballowitz, 1890, 1915b) showed that the axial filament is complex and consists of several fibrils. It was impossible to determine the precise number of fibrils since the thickness of an individual fibril is at the limit of resolution of the light microscope.

According to studies of sperm tails of various animals (including fishes) in polarized light (Pattri, 1932; Schmidt, 1935; Kirpichnikova, 1945), it was shown that the tails have a weak double light refraction of textural or structural anisotropy (Formdoppelbrechung), indicating that there are longitudinally arranged protein fibers in the sperm tail.

Ultramicroscopic studies of sperm tails, macerated or partially destroyed ultrasonically, as well as of sections, have confirmed that the axial filament is actually a bundle of thinner fibrils, remarkably uniform in structure in different animals and similar to the axial fibrillar complex of spermatozoids of plants, the protozoan flagellum and cilia of metazoan ciliated epithelium (see Fawcett and Porter, 1954; Bradfield, 1955; Fawcett, 1961; Burnasheva et al., 1963; Poglazov, 1965). The fibrillar complex is always constructed according to a single plan (see Figure 22 showing the structure of a sea urchin sperm tail); 11 fibrils run parallel in a column of cytoplasm: two thinner single fibrils (M-fibrils) in the center of the bundle and the remaining 9 doublets (L-fibrils) surround them in a ring.

Deviation from this plan among typical flagellates is very rare. In particular, spermatozoa with a fibrillar formula of 9 + 1 and 9 + 0 were described in the case of several worms (see Bonsdorff and Telkkä, 1965).

The sperm tails of many of the fishes studied (selachians, chimaeras, lungfishes, sturgeons and teleosts) have a typical (9 + 2) fibrillar complex. An exception is Lycodontis afer in which the fibrillar complex of the spermatid lacks central fibrils and belongs to the 9 + 0 type (Boisson et al., 1967a). In Squalus suckleyi and the chimaera Hydrolagus colliei (Stanley, 1965a) two supplementary longitudinal cords of large diameter (see p. 104) were found in addition to the fibrillar complex. A peculiar feature of spermatozoa of the lungfish Protopterus annectens is the presence of two flagella of typical structure (see p. 105).

The central M-fibrils of the axial fibrillar complex are distinguished from the outer L-fibrils by their properties. As shown in the case of gurnard spermatozoa, the M-fibrils are less sturdy, easily destroyed by distilled water and rapidly digested by pepsin (Grigg and Hodge, 1949). Therefore, on maceration 9 instead of 11 fibrils are often found (Bradfield, 1953). Apparently it is because of the lower resistance of the M-fibrils that only 9 fibrils were found on examination of spermatozoa of Coregonus wartmanni (Rõtheli et al., 1950).

The structure of the tail was studied in detail in the case of sea urchin spermatozoa (Afzelius, 1959). The subfibrils in the outer doublet L-fibrils differ somewhat in diameter (Figure 22). From the thicker subfibril of each pair emerge short outgrowths, which represent a transverse section of the lateral rib of the fibril. In addition, radial thickenings of the interfibrillar matrix were found running from the center of the complex to outer fibrils. In sections of the tail the subfibrils with their outgrowths are orientated in a clockwise direction in relation to the thinner subfibril of each pair; thus the fibrillar complex is asymmetrical.

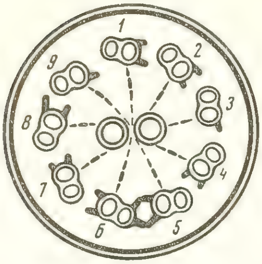

FIGURE 22. Schematic transverse section of tail of sea urchin spermatozoon (after Afzelius, 1959)

Of similar construction are the L-fibrils of bull spermatozoa and of the marine teleost of Calamus sp. (Gibbons, 1963); one of the subfibrils of each pair bears short outgrowths directed clockwise when viewed from the head toward the tail.

The individual fibril of the axial filament of the flagellum is a hollow cylinder or tube of complex structure, as shown by negative staining particularly in spermatozoa of insects and mammals (Pease, 1963; Phillips, 1966); the fibril wall consists of 10—13 filament-protofibrils, 40—70 Å in diameter.

It is not clear why the fibrillar formula of flagella and cilia is so remarkably constant. Bredfield (1955) suggested that the combination 9 + 2 evolved by chance and on proving to be a successful mechanical solution became established. Its preservation in a vast number of living organelles may be explained by the extremely small probability of a favorable mutation of the centrosome, the basal body responsible for flagellar structure.

Chemical analysis of sperm tails of salmon (Miescher, 1896) shows that their basic components (in%) are:

Protein	41.90
Lecithin	31.83
Fats, cholesterol	26.27.

Study of the sperm tails of perch, taimen and char (Tibbs, 1957, 1958) confirmed the presence of a large quantity of lipids, which is 40% in perch. The protein extracted from the tails does not belong to the collagen group. It contains 3.38—4.26% tyrosine, 2.63—3.06% tryptophan and 1.12% cystine (this calculation is for protein containing 16.0% nitrogen). The tails also contain 1.3—1.8% hexose-type monosaccharide. There are no nucleic acids. An analysis of Salmo fontinalis spermatozoa cytoplasm mainly containing tail material revealed 9.5% RNA (Felix et al., 1951); this discrepancy may be due to the fact that the preparation was not purely of sperm tails.

The cytoplasmic fraction and isolated sperm tails of teleosts show ATPase activity (Felix et al., 1951; Tibbs, 1957, 1958, 1962; Mohri, 1964).

2.2. SPECIFIC ORGANIZATION OF THE SPERMATOZOA IN DIFFERENT GROUPS OF FISHES

2.2.1. Selachians

Selachian spermatozoa are very large (Table 19): their total length usually exceeds 100μ and head length is 30–40μ (which is 10–20 times the length of sperm heads of most teleosts). The head is elongate-cylindrical or filiform with corkscrew flexures (Figure 23). The flexures of the head are expressed to a different degree in different species; for example, in Spinax niger (Figure 23, 3) they are scarcely discernible, but in Raja clavata (Figure 23, 6) they are very distinct. The number of flexures also varies considerably: 3 in Spinax, $5\frac{1}{2}$ in Raja clavata, 7–8 in Cetorhinus maximus and about 12 in Squalus acanthias (Figure 23) (Ballowitz, 1890; Retzius, 1909; Harrison Matthews, 1950). The distinctive head shape is determined by the spirally twisting supporting fiber, first discovered by Retzius (1902, 1909). In his opinion, this fiber is part of the plasmalemma of the spermatozoon, but according to the data of Kol'tsov (1909, 1936) it is located between the membrane and the nucleus.

Investigations of spermatogenesis in several selachian species (Grassé and Tuzet, 1932; Tuzet and Cabanis, 1959; Tuzet, 1950a, 1958) led to the conclusion that the supporting fiber is a derivative of the proximal centriole. It may twist around the nucleus externally (as in Scyliorhinus caniculus) or else pass within the nucleus from the base, where the proximal centriole is located, to the acrosome (Mustelus mustelus, M. canis, Squalus acanthias, Raja clavata); see Figure 24.

The sperm head terminates anteriorly in a thin pointed acrosome, According to the above investigations of French cytologists, there is in front of the nucleus also an "anterior centrosome" (named to distinguish it from the "posterior centrosome" which includes the proximal and distal centrioles forming the middle piece). During spermatogenesis the "anterior centrosome" in contact with the acroblast moves to the future anterior pole of the nucleus; in subsequent stages it is located either at the base of the acrosome (in Scyliorhinus caniculus; Figure 24, a, b) or else at its tip (Mustelus mustelus, M. laevis, Squalus acanthias, Raja clavata; Figure 24, d, f). From it grows a forward-directed cilium (stéréocil; see Figure 24, g, h) which, when the spermatozoon is in the testis, penetrates the cytoplasm of Sertoli cells and serves to attach to it. The nucleus is covered externally by a lipid membrane.

The presence of more than two centriole elements in the spermatozoon is possible in the light of recent data that during interkinesis each of the two centrioles already has dual organization (see Goroshchenko, 1965). However, ultramicroscopic investigation is required to assess accurately the formations described as the "anterior centrosome" and "stéréocil."

The middle piece is cylindrical. Through its center passes an axial rod which passes into the axial filament of the tail (Figure 23, 2 and 4).

The position of the distal centriole or its derivatives is undoubted; a clearly discernible ring-shaped body (B. Suzuki, 1899; Retzius, 1902, 1909; Stephan, 1904; Grassé and Tuzet, 1932; Tuzet and Cabanis, 1959) or round granule (Vasisht, 1954a), from which the tail flagellum emerges, mark the posterior boundary of the middle piece.

TABLE 19. Dimensions of fish spermatozoa (in μ)

Subclass, family	Species	Total length of spermatozoon	Head dimensions		Author
			length	width	
Elasmobranchii					
Cetorhinidae	Cetorhinus maximus		40	0.4—0.5	Harrison Matthews, 1950
Scyliorhinidae	Scyliorhynus caniculus	~160	30		Metten, 1939
		100*	29*	~1*	Tuzet and Cabanis, 1959
Carcharinidae	Mustelus mustelus	75*	34*	~0.6*	Tuzet and Cabanis, 1959
	Mustelus laevis (M. canis)	73*	22*	~0.5*	Tuzet and Cabanis, 1959
Squalidae	Squalus acanthias	126*	34	~0.5*	Tuzet and Cabanis, 1959
Rajidae	Raja clavata	215	50		Ballowitz, after Waldeyer, 1906
		113*	35*	~0.5*	Tuzet and Cabanis, 1959
Dipnoi					
Lepidosirenidae	Protopterus annectens	~80	40	~3.0*	W. N. Parker, 1892
		180—200	30—35	3.0—3.5	Boisson, 1963
Teleostomi					
Coelacanthidae	Latimeria chalumnae	~90*	28.8		Tuzet and Millot, 1959
Acipenseridae	Acipenser sturio	49.5	4.5	1.8	Ballowitz, 1890
	Acipenser güldenstädti colchicus	58	8.9	1.9	Detlaf and Ginzburg, 1954
	Acipenser stellatus	47	6.3	1.8	Detlaf and Ginzburg, 1954
	Huso huso	55	7.4	1.1	Detlaf and Ginzburg, 1954
Polyodontidae	Polyodon spathula	~20			Larimore, 1950
Clupeidae	Clupea harengus pallasi	43	2.0	1.5	Yanagimachi, 1957b
	Clupea harengus membras	64.5—77.5	2.5	2.0	Kupffer, 1878
Salmonidae	Oncorhynchus kisutch		2.0	1.5	Lowman, 1953
	Oncorhynchus tschawytscha	19.6	2.6	2.0	iddle, 1917
	Salmo salar	63*	4—4.5	3.5—4	His, 1873
	Salmo trutta m. lacustris	31—34	2.0—2.4	1.5—2.0	Ginzburg, unpublished
	Coregonus lavaretus wartmanni	43.0—48.2	3.0—3.2	2.0—2.7	Rötheli et al., 1950
	Coregonus lavaretus asperi	43.5	3.5	3.0	Rötheli et al., 1950
Esocidae	Esox lucius	43.6	2.2	2.2	Ballowitz, 1890
		20—25	1.5	1.5	Lindroth, 1946
		37—42	2.0	2.0	Rötheli et al., 1950
Cyprinidae	Carassius carassius	17.7	3.2	3.2	Kobayasi, 1963
	Rhodeus ocellatus	39	2.6	2.2	R. Suzuki, 1959a
Cobitidae	Misgurnus anguillicaudatus	21.5	3.0	2.8	Kobayasi, 1963
Anguillidae	Anguilla anguilla	11	5	~0.7*	Tuzet and Fontaine, 1937
Cyprinodontidae	Poecilia (Lebistes) reticulata	59	4.2	1.3	Ginzburg, unpublished
	Xiphophorus helleri		2.55	1.35	Essenberg, 1923
		60	3.6	1.3	Ginzburg, unpublished
Cottidae	Oligocottus rubellio	53.0	5.5	1.1	Fink and Haydon, 1960
	Oligocottus snyderi	38.6	4.0	0.8	Fink and Haydon, 1960

* Spermatozoan dimensions changed according to illustrations provided by the authors.

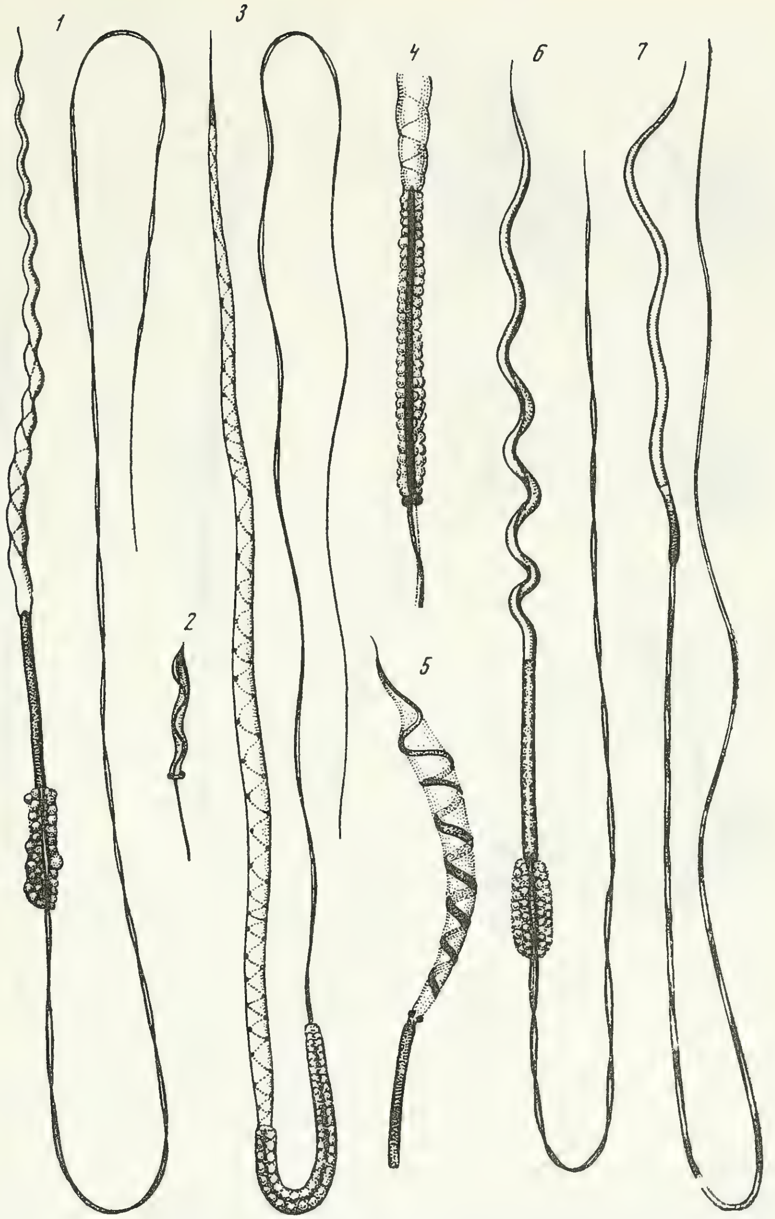

FIGURE 23. Spermatozoa of selachians and chimaeras: *Squalus acanthias*(1,2), *Spinax niger* (3,4), *Raja clavata* (5,6) and *Chimaera monstrosa* (7):

1,3,6,7 — unchanged spermatozoa; 2 — isolated middle piece with intensively stained axial rod and ring centriole; 4 — middle piece with axial rod covered by granular mitochondrial sheath; 5 — swollen head with clearly discernible skeletal fiber (after Retzius, 1909).

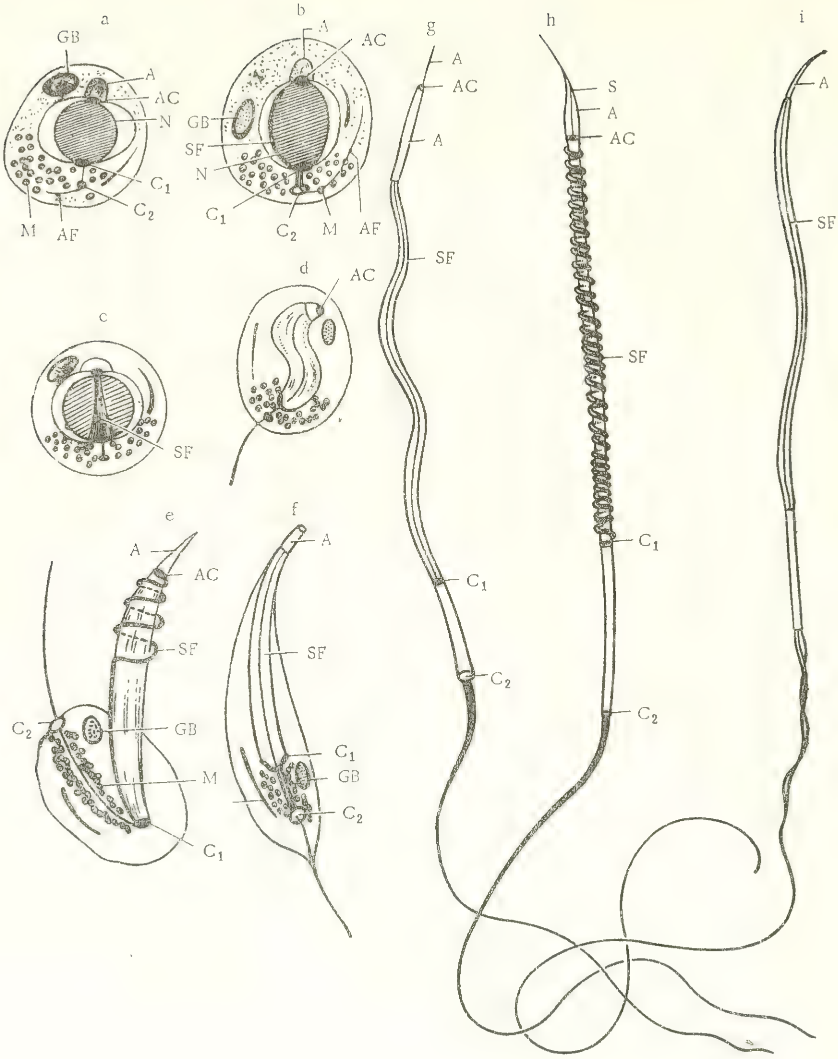

FIGURE 24. Spermatogenesis and mature spermatozoa in selachians and Latimeria:

a, b, e, h — Scyliorhinus caniculus (a, b, e — transformation of spermatid, h — spermatozoon); c, d, f — Squalus acanthias (transformation of spermatid); g — Raja clavata (spermatozoon); i — Latimeria chalumnae (spermatozoon) (after Tuzet, 1958). A — acrosome; M — mitochondria; GB — Golgi body; SF — supporting fiber; AF — axial filament of tail; AC — anterior centrosome; S — stéréocil; C_1 — proximal centriole; C_2 — distal centriole; N — nucleus.

Data on the position of the proximal centriole and the origin of the axial rod of the middle piece are inconsistent. According to some investigators (Grassé and Tuzet, 1932; Tuzet and Cabanis, 1959, see Figure 24), the axial rod arises from the desmose which links the proximal and distal centrioles at the time of their separation. In the mature spermatozoon the proximal centriole is located at the anterior boundary of the middle piece at the base of the nucleus, while the distal centriole is located at the boundary between the middle piece and the tail. On the other hand, according to Suzuki (1899) and Stephan (1904), the axial rod of the middle piece is a derivative of the proximal centriole. Even when at the periphery of the young spermatid, this centriole grows in the direction of the nucleus and turns into an axial rod; accordingly, the proximal centriole cannot be seen during formation of the spermatozoon. Vasisht (1953, 1954a) also concluded that the axial rod is derived from the proximal centriole, but that this rod grows after the proximal centriole approaches the nucleus, and in the mature spermatozoon this centriole is preserved at the anterior boundary of the middle piece in the recess of the head. However, electron micrographs of spermatozoa of Squalus suckleyi showed both centrioles, the proximal and the distal, at the posterior end of the middle piece (Stanley, 1964, 1965a). This confirms the idea that the axial rod of the middle piece is formed by the proximal centriole. Nevertheless, the position of the proximal centriole may vary in different species of selachians.

The axial rod is covered externally by a mitochondrial membrane, whose granular structure is conspicuous even in immature spermatozoa; sometimes individual mitochondrial elements of mature spermatozoa are even discernible under a light microscope (Figure 23, 3, 4). Electron micrographs of spermatozoa of Squalus suckleyi show that the axial rod is surrounded by densely packed circular mitochondria (with concentric arrangement of intramitochondrial membranes) and an outer sheath of thin helical filaments or tubules (Stanley, 1964).

A cytoplasmic connecting piece, enveloping the base of the axial filament, is located where the middle piece passes into the tail (Figure 23, 1, 6). This connecting piece is formed from granular cytoplasm in the last stages of spermatogenesis (Mellinger, 1965), is easily damaged and is often lost during cytological investigations (in particular, it is absent in the spermatozoon of Spinax niger shown in Figure 23, 3).

The distinctive structure of the tail of the selachian spermatozoon was already noticed in early cytological investigations (Jensen, 1879, 1883; Ballowitz, 1890; Retzius, 1902, 1909); the tail is a coiled flat band along whose edges pass two identical filaments. Upon maceration, the filaments move apart, do not divide into thinner fibrils (Retzius, 1902). Electron micrographs of spermatozoa of Squalus suckleyi (Stanley, 1965a) showed that the paired filaments or cords described earlier are supplementary formations of complex structure, lying at the sides of the typical axial fibrillar complex (9 + 2 fibrils). In the center of each cord there is a filament strewn with granules and enclosed in a thick-walled sheath of dense material. Both cords emerge from the distal ring surrounding the distal centriole and pass along the entire tail in a double helix twisting around the axial fibrillar complex.

The sperm tail in selachians becomes gradually thinner toward the end; there is no separate end piece.

2.2.2. Chimaeras

The spermatozoa of chimaeras are formed similarly to those of selachians (Stephan, 1903) and resemble them in structure (Retzius, 1909). The spermatozoon of Chimaera monstrosa (Figure 23, 7) has a long cylindrical head describing about 3 spirals and ending in a thin pointed acrosome. The supporting fiber runs in the spiral recesses, but its boundaries are less distinct than in those of supporting fibers of selachian spermatozoa. The mitochondrial membrane of the middle piece has an indistinct granular structure; at its end a double distal ring can be discerned. There is often a cytoplasmic connecting piece, at the boundary with the tail, which in some cases envelops the middle piece and sometimes even the caudal end of the head. This connecting piece is easily destroyed. The sperm tail twists spirally, becomes gradually thinner toward the end and does not form a special end piece.

Electron micrographs of spermatozoa of Hydrolagus colliei showed that the axial complex of the tail has a typical 9 + 2 fibrillar formula (Stanley, 1965a). As in Squalus suckleyi, two supplementary cords also arise in the tail of chimaeras but one is very short and found only near the distal centriole. The second additional cord extends along the entire tail. This cord and the axial fibrillar complex together form a double helix, neither occupying a central position.

2.2.3. Lungfishes

Spermatozoa of Protopterus annectens have an elongated head with a pointed anterior end; adjoining the posterior end of the head is a short hemispherical middle piece (W. N. Parker, 1888, 1892; Boisson, 1963; see Figure 25, a).

A distinctive feature of the Protopterus spermatozoon, first noted by Parker (1888, 1892) is the presence of two unconnected caudal flagella. These flagella (Boisson, 1963) reach a length of about 150μ and are of similar structure. Electron micrographs show that each flagellum has a typical fibrillar complex (9 + 2). The cytoplasmic membrane of the flagellum forms two lateral outgrowths, giving it a ribbon shape, which are narrower at the base and widen toward the tail end where the tail section is 2μ wide (Figure 25, b, c).

The nucleoplasm is covered at the tip of the sperm head by the acrosome. Studies of spermatogenesis in Protopterus (Boisson and Mattei, 1965; Boisson et al., 1967b) did not reveal the usual stage of acrosome formation, that of the acrosomal vacuole enclosing a granule. The anlage of the acrosome appears as a thin, slightly curved platelet 2–3μ in width, which closely adjoins the nuclear membrane and has the form of a Golgi cisterna with closely approximated walls (Figure 26, a). Subsequently the acrosomal anlage becomes cup-shaped, envelops the distal end of the spermatid nucleus and, later, in the mature spermatozoon, is shaped like a hollow pointed cone (Figure 26, b).

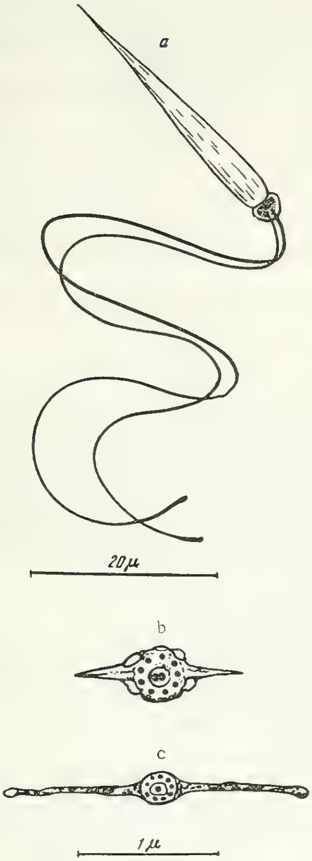

FIGURE 25. Spermatozoon of Protopterus. General view (a), sections of tail at base of flagellum (b) and farther caudal (c) (after Boisson, 1963).

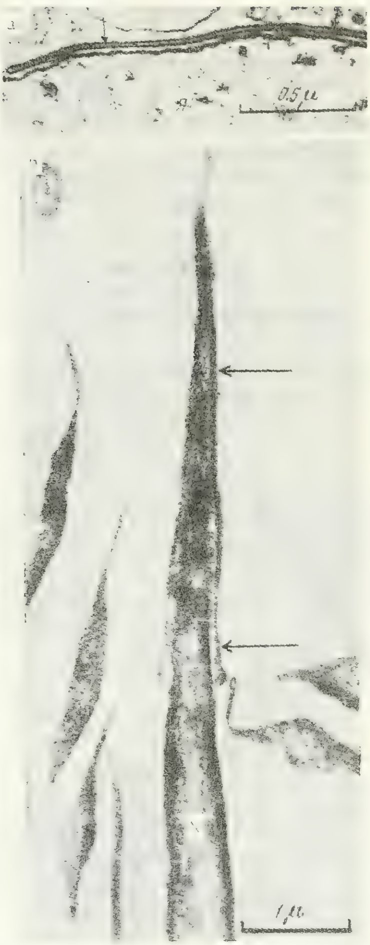

FIGURE 26. Acrosome of Protopterus spermatozoon (electron micrographs):

a — anlage of acrosome (indicated by arrow) in young spermatid; b — sperm-shaped acrosome (indicated by arrows) in mature spermatozoon (after Boisson and Mattei, 1965). N — nucleus.

As in other animals, two centrioles participate in formation of the middle piece. Contrary to what is usually observed during spermatogenesis, these centrioles are not differentiated into proximal and distal elements, but are arranged 1μ apart, parallel to the periphery of the spermatid; from each centriole arises a separate caudal flagellum (Boisson et al., 1967b).

2.2.4. Crossopterygian Fishes

Spermatozoa of Latimeria chalumnae have about the same dimensions as those of selachians (Table 19) and show many similar features (Tuzet and Millot, 1959; see Figure 24). Thus the sperm head of Latimeria is elongate-cylindrical and ends in a pointed acrosome. As in spermatozoa of Squalus acanthias, Mustelus mustelus and several other selachians, the head is pierced by an intranuclear supporting fiber, which grows from the proximal centriole. The middle piece is cylindrical.

The anterior part of the tail of the Latimeria spermatozoon is furnished with a short undulating membrane.

2.2.5. Chondrostean Fishes

Among chondrostean fishes, spermatozoan structure is known only for representatives of two sturgeon genera, Huso and Acipenser; spermatozoa of Acipenser sturio (Ballowitz, 1890), A. güldenstädti colchicus, A. stellatus and Huso huso (Detlaf and Ginzburg, 1954; 1963; Ginzburg, unpublished) have been investigated. Sturgeon spermatozoa are much smaller than those of selachians and the lungfish Protopterus, but of the same size as those of teleosts, having a total length of about 50μ (see Table 19).

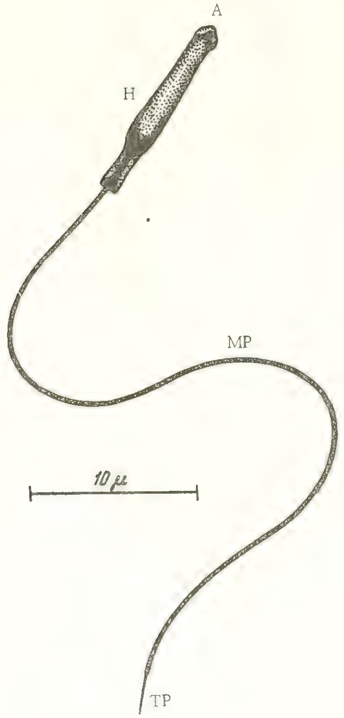

FIGURE 27. Diagram of sturgeon spermatozoon:

A — acrosome; H — head and middle piece; MP — principal piece of tail; TP — end piece.

The sperm head (Figure 27; Plate V, a) is rod-shaped, with a somewhat widened posterior part. The acrosome at the anterior part is shaped like a cap with a rounded top. The nucleoplasm forms a small process which protrudes into the acrosome.

Adjoining the head is a cylindrical middle piece which in spermatozoa of Acipenser güldenstädti colchicus, A. stellatus and Huso huso, according to my observations, has a much smaller volume than the middle piece in spermatozoa of Acipenser sturio, as described by Ballowitz. This is not likely to be a specific structural feature, but rather a secondary swelling of the middle piece in the spermatozoon of A. sturio. It is also possible, that Ballowitz observed immature spermatozoa, since he examined testical contents during the spawning period and not fluid products.

The proximal axial bundle in the middle piece terminates anteriorly at the centriole at the base of the head, whereas the posterior boundary of the middle piece is a very large bowl-shaped formation with the opening facing forward (Ballowitz, 1890), apparently the distal ring.

The mitochondrial sheath of the middle piece envelops the axial filament evenly on all sides as in selachians (but not as in most teleosts where the arrangement is asymmetrical). This was observed in electron micrographs of spermatozoa of Acipenser güldenstädti colchicus fixed according to Palade's technique (Plate V, a).

The tail is 6—7 times longer than the rod-shaped head. There is a distinct end piece (Plate V, c), 1.8—2.7 μ in A. sturio (Ballowitz, 1890) and 2.1— 3.0μ in A. güldenstädti colchicus (Ginzburg, unpublished). Ballowitz established that the axial filament of the sturgeon sperm tail has a complex structure. He counted 5 fibrils in macerated tails, indicating that some fibrils are thicker than others and apparently consist of even thinner subfibrils. My ultramicroscopic studies of tail pieces of sturgeon spermatozoa indicate that the axial complex has the usual structure.

2.2.6. Holostean Fishes

The spermatozoa of the bowfish Amia calva (Retzius, 1905), similar in size to those of teleosts (Figure 28, 1), have spherical or oval heads with no discernible acrosome. The relatively large hemispherical middle piece adjoining the end of the head has indistinct granularity. The axial filament passes medially through the middle piece. The tail narrows toward its end without distinct definition of an end piece.

2.2.7. Teleosts

Among fishes, teleost spermatozoa have the simplest structure; they are similar in size to those of sturgeons (Table 19). In most teleosts studied, the sperm head is 2—3μ long and the total length of the spermatozoon 40—60μ.

The head is usually subspherical, but rod-shaped and elongated heads are also found (Ballowitz, 1890, 1915a, b; Retzius, 1905; Hann, 1927, 1930; Rötheli et al , 1950; Lowman, 1953; Fink and Haydon, 1960; Ginzburg, 1963b).

A spherical head has been described for many species, e. g., carp and pike (Figure 28, 2, 3). This should be regarded with caution, however, since increased salinity of the solution and various chemical effects can easily cause heads to swell. This explains why salmon and herring sperm heads have been described as spherical (Retzius, 1905), while mature spermatozoa actually have a very regular geometrical shape when not affected by external conditions, and are cordate or acorn-shaped and somewhat flattened laterally (Ballowitz, 1915a, 1915b). However, unaffected pike sperm heads are regularly spherical (Ballowitz, 1890; Rötheli et al., 1950, see Figure 29); the same applies to spermatozoa of Pelecuo cultratus and bream (Figure 30, d, e).

Quite often a slight deviation from spherical is encountered — oval (burbot), ovate or cordate (Gobius niger, Figure 28, 6, 7; Salvelinus alpinus = Salmo salvelinus, silver salmon, Clupea harengus), and also acorn-shaped (Coregonus wartmanni, brook and lake trout, Figures 28, 10 and 30, a). Less frequently the sperm heads are elongated (guppy, swordtail, Plate V, d; several Cottidae, Figure 31, c, d). In some species the head is slightly flattened laterally (e. g., in perch, Figure 28, 4, 5; herring, and trout). Sometimes the width of the head varies so greatly in different cross sections that the head resembles a disc (Zoarces viviparus, Figure 20, 8, 9) or has a flattened-oval shape; moreover, one side of the head may be somewheat concave and the other side convex (Cottidae, Figure 31).

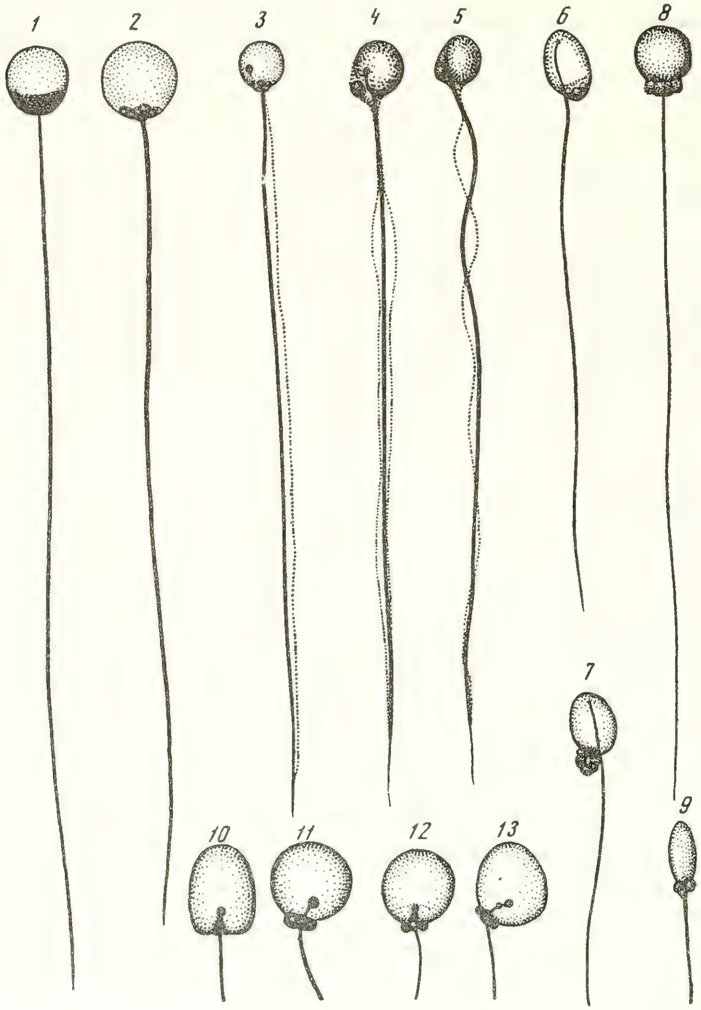

FIGURE 28. Spermatozoa of bowfish (1) and teleosts: carp (2), pike (3), perch (4, 5), Gobius niger (6, 7), viviparous blenny (8, 9) and brook trout (10 — unaffected head, 11—13 — swollen heads stained with gentian violet) (1—9 after Retzius, 1905; 10—13 after Ballowitz, 1915b).

The shape of the sperm head, as a rule, hardly varies within a family but differences have been established in the Cottidae family which exceed those for all other teleosts. Among 32 species studied in this family (Hann, 1930), oval and, to varying degrees, elongated heads (e. g., Figure 31, a–d), have been described which show a distinct correlation to the phylogenetic position of the species in the Cottidae; thus, the degree of kinship between any two species, subgenera or genera in this family can be determined by the degree of similarity of their spermatozoa (Fink and Haydon, 1960).

As mentioned, no acrosome was found in spermatozoa of most teleosts studied (e. g., herring, pike, carp, salmon, trout, burbot, perch, goby, Lepomis, etc.), either by standard cytological methods (Ballowitz, 1890, 1915a, b; Retzius, 1905; Kol'tsov, 1909) or in using PAS reaction in cytochemical investigations, which selectively stains mammalian and insect acrosomes (Cavazos and Melampy, 1951) or in electron micrographs of entire spermatozoa in seminal fluid (Fisher et al., 1952; Lowman, 1953; Fink and Haydon, 1960; Stanley, 1965b) and spermatozoa induced to eject the acrosomal filament (Ginzburg, 1963a, b).

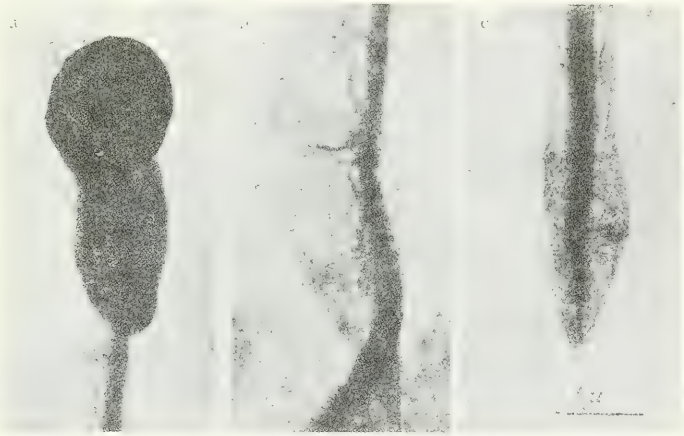

FIGURE 29. Pike spermatozoon:

Palade's fixation. Electron micrographs: a — sperm head with middle piece; b — principal piece of tail; c — end piece.

The data differ somewhat on the presence of an acrosome in spermatozoa of platys and eels.

In a work devoted to spermatogenesis in 3 species of platys — guppy, swordtail and gambusia (Gambusia wrayi) — Vasisht (1954b) described formation of the acrosome. Accordingly, the acrosome is a very conspicuous conical structure located before the nucleus in the head, clearly discernible in sections of the testis and in live mature spermatozoa in both light and phase microscopes.

However, the existence of an acrosome in platys' spermatozoa was not confirmed by other investigators. No acrosome was described in the first cytological work devoted to spermatogenesis in the guppy (Vaupel, 1929), nor in spermatids or spermatozoa of guppy and swordtail in the phase microscope, with fluorochrome treatment with acridine orange or with PAS-reactions in sections of testes (Ginzburg, unpublished). Conclusive evidence of Vasisht's error was furnished by an ultramicroscopic study of spermatogenesis in the guppy (Porte and Follenius, 1960), which showed that in this species the spermatozoon lacks an acrosome and that no signs of an anlage could be found in spermatids; the Golgi body and rebundant cytoplasm are discarded at initial stages of spermatogenesis and do not participate in forming the anterior section of the spermatozoon.

Thus, spermatozoa of platys, like those of other teleosts, lack acrosomes with the sole exception of Anguilla anguilla (Tuzet and Fontaine, 1937; Tuzet, 1950a, 1958). Owing to insufficient material, it was impossible in a study of spermatogenesis of this species to trace the consecutive stages in acrosomal formation. However, the authors did observe fusion of dictyosomes into a structure resembling an acroblast and a spear-shaped protrusion, about 2 μ long, at the end of the heads of secondary spermatids and mature spermatozoa, which stained like acrosomes of the spermatozoa. Ultramicroscopic studies of spermatogenesis in the eel should be interesting.

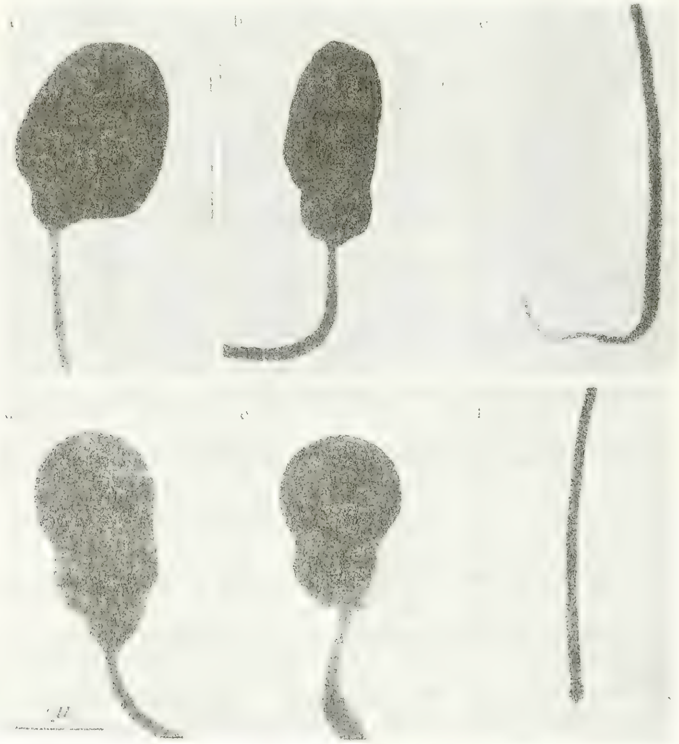

FIGURE 30. Spermatozoa of lake trout (a), Caspialosa tanaica (b, c), Pelecus cultratus (d) and bream (e, f).

Fixed with 4% formalin in Ringer's solution. Electron micrographs. a, b, d, e — head with middle piece; c, f — end of tail.

In many teleost species, the base of the spermatozoan head has a rather deep recess in which two centriole bodies can be observed. Thus, when the heads of Salmo trutta m. fario (Ballowitz, 1915b) spermatozoa swell, two centrioles, one immediately behind the other, are revealed (Figure 28, 12, 13).

Ultramicroscopic study of spermatogenesis in two platys, Cnesterodon decemmaculatus (Sotelo and Trujillo-Cenóz, 1958) and Poecilia (Lebistes) reticulata (Mattei and Boisson, 1966), fully proved that two typical centrioles participate in forming the middle piece. In the early spermatid, the centrioles are located at the cell periphery perpendicular to the cell membrane and at an acute angle to one another (Plate VI, a); as they approach one another, the proximal centriole forms a right angle to the distal centriole which initiates the axial complex. The centriolar complex moves toward the nucleus, penetrating a concavity which developed in it (Plate VI, b) and which by the end of spermatogenesis is transformed into a tubule enclosing centriole apparatus and the beginning of the tail flagellum (Plate VI, c).

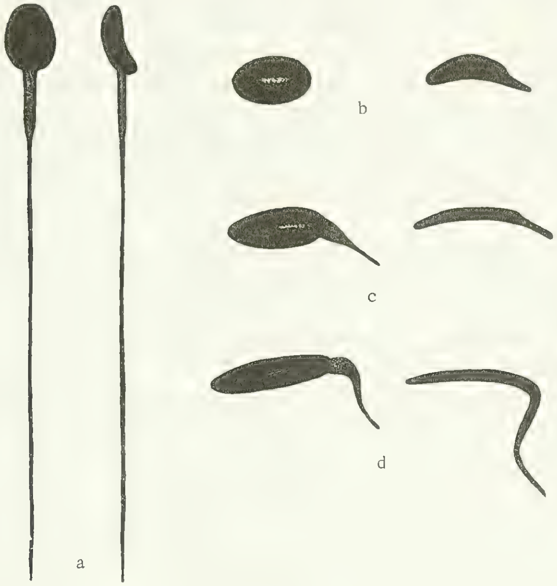

FIGURE 31. Spermatozoa of Cottidae:

View of head from flattened side and laterally. a — Cottus bairdii; b — Ascelichthys rhodorus; c — Axyrias harringtoni; d — Blennicottus globiceps (after Hann, 1930).

The centrioles in Cnesterodon are cylindrical bodies about 0.26 μ in diameter, whose walls consist of fibrils or tubules about 130 Å wide surrounded by dense material. The space between the two centrioles is filled with thin fibrils emerging from the distal centriole; transverse discs are also discernible in this space.

In the guppy, the proximal centriole gives rise to a conical formation of fibrillar structure which occupies the anterior, narrowed part of the ultranuclear canal (Plate VI, c); this formation resembles the axial body ("corps axial") of bivalve mollusk spermatozoa (see Galtsoff and Philpott, 1960; Bourcart et al., 1965). Behind it are located the proximal centriole, (0.5 μ in length), perpendicular to the head axis, the intercentriole formation with several osmiophilic discs and the distal centriole (0.7 μ long) extending into the axial complex. Four osmiophilic "satellites" are arranged around the distal centriole. Both centrioles have the typical cylinders formed by 9 groups of fibrils, each consisting of 3 fused subfibrils.

In some species (Esox lucius, Perca fluviatalis, Pleuronectes flesus) the centriole body lies on the lateral surface of the head. The axial filament passes from this body under the head plasmalemma to the posterior of the head where it independently forms the tail (Figure 28, 3, 4). Only rarely (in Gobius niger, Figure 28, 6, 7) is the centriole located almost at the very anterior end of the head.

In most teleost spermatozoa, the middle piece is a small spherical or flattened formation, called the "tail globule" by N. K. Kol'tsov (1909), which adjoins the base of the head and often (e.g., in salmonids) extends into a concavity there. The axial filament passes through the tail globule eccentrically, sometimes along the very edge of it (Ballowitz, 1890, 1915b; Retzius, 1905; Kol'tsov, 1909). In silver salmon spermatozoa (Lowman, 1953) the tail globule merely touches the head, but is not connected to it, and is joined to the base of the axial filament laterally. In cases where the axial filament emerges from the lateral surface of the head, and not its base, the middle piece plasm is distributed in a thin layer on its surface (Retzius, 1905).

The first cytological investigations of mature spermatozoa (Retzius, 1905, 1910; Ballowitz, 1915b, 1916) with aniline stains (gentian violet, rosaniline) revealed round, intensely stained granules, usually numbering 4 or 5, in the tail globule. For example, 4 rather large spherical formations are very clearly visible at the head base of Lophius piscatorius spermatozoa. In some cases, the number of granules is smaller (only one large granule in perch) or greater (in herring, viviparous blenny, etc.). The spermatozoon of Idus melanotus is exceptional in that the middle piece contains more than twenty such granules.

Study of spermatogenesis in Fundulus (Dusber, 1918) established that these granules are modified mitochondria. In the spermatid, mitochondria (usually about 8) concentrate at the caudal end of the nucleus and surround the base of the axial filament; then they must fuse, since their sizes increase and numbers decrease generally to 3–4. Thus, the homology between granules of the tail globule in the teleost spermatozoon and the perinuclear body (Nebenkernorgan, Nebenkern) of coelenterate, worm, mollusk and insect spermatozoa, which was mentioned preliminarily (Retzius, 1905; Wilson, 1925), is fully confirmed; both the perinuclear body (Meves, 1900; Bowen, 1922a; De Robertis and Franko Raffo, 1957; Kaye, 1958a; Gatenby and Dalton, 1959; Novikoff, 1916) and the teleost middle piece granules are modified mitochondria.

Unlike the helical arrangement of middle piece mitochondria in selachians, the few large mitochondria of the teleost tail globule are distributed irregularly forming a compact mass.

Not all teleosts have the above middle piece structure. In certain families it is much more strongly developed with the axial filament in its center. In Cottidae, particularly, the middle piece is about as long as the head (Fink and Haydon, 1960), while in platys it is even longer (see Plate V, d). The middle piece in the guppy (Porte and Follenius, 1960) has a distinctive structure. The axial filament passes through the center of the middle piece, which is entirely covered by a plasmalemma (which thus forms a deep fold). The large modified mitochondria are swollen, their crests are shifted toward the periphery and lie in concentric circles. The mitochondria do not fuse into a single helix.

The middle piece of the swordtail has similar structure (Ginzburg, unpublished data); the modification of the mitochondria is clearly discernible in Plate VIII.

The tail of most teleost spermatozoa (herring — see Figure 30, c, salmon, trout, silver salmon, roach, burbot, perch, ruffe, plaice, etc.) has a clearly pronounced end piece. The tail diameter remains constant throughout the principal piece, then narrows sharply at the transition to the end piece. In silver salmon spermatozoa, the diameter of the principal piece is about 1,600 Å, decreasing to 1,000 Å at 2–3 μ from the end and to 300–600 Å 1 μ nearer the end (Lowman, 1953).

In some fishes, e. g., pike (Figure 29, c), carp, goby, silver salmon, guppy and swordtail (Plate V, f), the tail has no pronounced end piece and gradually thins toward the end; the bream spermatozoan tail remains the same diameter up to the very tip (Figure 30, f).

It was noted earlier that the axial filament in tails of spermatozoa from various animals consists of 11 fibrils, 2 inner and 9 outer, covered by a cytoplasmic sheath. The same structure is found in teleost spermatozoan tails, e. g., in silver salmon (Lowman, 1953), carp (Yasuzumi, 1956), loach (Ginzburg, unpublished) and guppy (Mattei et al., 1967a). The only known exception is the spermatozoon of Lycodontis afer (Murenidae) which has a fibrillar formula of 9 + 0 (Boisson et al., 1967a).

The individual fibrils are clearly discernible on maceration in distilled water of the tail flagellum of loach (Plate VII). It can be seen in electron micrographs that the pair of inner M-fibrils are thinner than the outer 9 L-fibrils, and are easily destroyed by distilled water, like M-fibrils in gurnard spermatozoa (Grigg and Hodge, 1949).

In silver salmon spermatozoa, each fibril remains the same diameter throughout its length (Lowman, 1953). However, the fibrils differ in length; 7 of the 9 outer fibrils terminate at the end of the principal piece and the two others 1 μ closer to the end of the flagellum, whereas only the 2 inner M-fibrils reach the very end.

In platys, according to available data (Mattei et al., 1967a), the inner fibrils terminate shorter than the outer fibrils, which change sharply from doublets to single fibrils at the end of the tail. This was accomplished in various ways in the species studied. In spermatozoa of swordtail and mollies, one subfibril of each outer doublet is reduced. In guppy spermatozoa, the subfibrils of each doublet diverge, forming 18 separate fibrils; these apparently terminate at different levels, since consecutive tail sections show 17, 16, 15. . . . . 4, 3, and 2 fibrils toward the end of the tail. Accordingly, the spermatozoan tail of the guppy does not have a pronounced end piece.

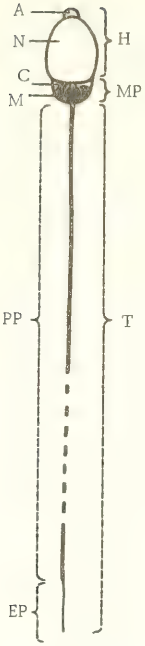

FIGURE 32. Diagram of primitive metazoan spermatozoon (after Franzén, 1956):

A — acrosome; H — head piece; PP — principal piece of tail; EP — end piece; M — mitochondrial body; MP — middle piece; T — tail section; C — centriole; N — nucleus.

The cytoplasmic sheath of the silver salmon spermatozoan tail is helical; there are 12—15 spiral ribs (Lowman, 1953).

A simple undulating membrane found in the tail of spermatozoa of a few teleost species (pike, ruffe, perch) — Figure 28: 3—5; Figure 29 (Ballowitz, 1890; Retzius, 1905; Rötheli et al., 1950) is most strongly developed in pike and less pronounced in perch. The undulating membrane reaches the end of the tail in pike spermatozoa which lack a distinct end piece; in ruffe and perch, it reaches only the end of the principal piece. The membrane of the pike spermatozoon (Rötheli et al., 1950; Ginzburg, unpublished) is a delicate cytoplasmic film 1 μ wide and approximately 70 Å thick with a somewhat thickened free margin. It is not clear whether it is attached to one side of the tail or winds around it. The undulating membrane in pike is easily disintegrated and disappears completely 4 minutes after the spermatozoon enters the water.

When spermatozoa of salmonids (Salmo irideus, S. trutta m. fario, S. fontinalis) are placed in hypotonic solutions or water, the cytoplasmic sheath of the tail rapidly swells entirely or in isolated vacuoles, giving the appearance of the undulating membrane of the pike spermatozoon (Fisher et al., 1952; Hug et al., 1953). If the spermatozoa are retained in hypotonic medium, cytoplasmic vacuoles of various sizes detach from the tails and float freely, sometimes in masses and chains.

Some investigators (Rötheli and Roth, 1950) have named these "kinoplasmic vacuoles" and attributed a role in spermatozoan movements to them. This suggestion has no basis since similar changes occur in other cells, e. g., blood cells, in hypotonic solutions (Hug and Lippert, 1951; Hug et al., 1953), and vacuoles are not formed by spermatozoa moving in isotonic solutions of sodium chloride, mannitol or saccharose, but appear on swelling of fixed, nonmotile spermatozoa (Lowman, 1953; Geiger, 1955).

The resemblance of the swollen cytoplasmic sheath of the axial filament of salmonid spermatozoa to the undulating membrane in pike was the basis for asserting that the latter membrane is an artifact of initial stages of plasmolysis (Hug et al., 1953). However, this opinion is argued against by the morphological features of the membrane; it is always located at one side of the axial filament, has a definite width and is regularly found in fixed preparations of spermatozoa from seminal fluid (Ballowitz, 1890) or freshly ejected into the water (Rötheli et al., 1950).

A very special case, not only among teleosts but also among all chordates, is that of Gymnarchus niloticus whose spermatozoa lack flagella (Mattei et al., 1967b).

2.2.8. Comparison of Spermatozoan Structure of Different Groups of Fishes

An analysis of known data indicates similar and varying features of spermatozoan structures of various fishes.

Spermatozoa of fishes which have retained external fertilization have many of the features inherent in flagellate spermatozoa of a primitive type. The latter are characterized (Franzén, 1956, see Figure 32) by a round or conical head, a small short middle piece containing several (4–5) mitochondria and a filamentous tail about 50μ long with a thin end piece; the head is comprised mainly of the nucleus and usually has a small cup-shaped acrosome. Such spermatozoan structure is found in many animals — coelenterates, sponges, worms, mollusks, crustaceans, echinoderms, enteropneusts, tunicates, cephalopods — which have ejection of sexual products into the water in common. The primitiveness of this type of spermatozoon is indicated by its wide distribution among systematically distant groups of animals and its occurrence as a stage in spermatogenesis in spermatozoa of divergent structure.

The sturgeon spermatozoon, like other primitive-type spermatozoa, has a cup-shaped acrosome, a short middle piece and a tail about 50μ along with a clearly pronounced end piece. However, the sperm head has been secondarily modified to a long rod shape; this is probably due to the development of dense, thick egg membranes pierced by narrow micropylar canals.

Most teleost spermatozoa have retained the primitive spherical or subspherical head, but have lost the acrosome. Reduction of the acrosome during evolution was possible because the spermatozoon comes into direct contact with the ooplasm deep in the micropylar canal; thus, the acrosome, as an instrument for penetrating the sperm-permeable barrier between the gametes, lacks significance. The middle piece in teleost spermatozoa is short and generally contains 4–5 mitochondria at the base of the nucleus, which is characteristic for the primitive type of structure (Franzén, 1956; André, 1962). Bradfield (1955) stated that herring spermatozoa were primitive, indicating that mitochondria of the middle piece form a compact mass covering only 1–2% of the axial filament length (whereas more advanced spermatozoa have a much more strongly developed mitochondrial sheath). Unlike many primitive invertebrate spermatozoa in which the mitochondrial sheath is wound helically around the axial filament, the mitochondria in teleosts are arranged randomly and the axial filament generally passes eccentrically through the middle piece. The tail usually has a distinct end piece. Many teleost spermatozoa also have a tail length similar to that of the primitive invertebrate spermatozoa.

Unlike the primitive-type spermatozoa, those of teleosts with internal fertilization — guppy, swordtail, etc. — have an elongated head and a well developed middle piece with a thick mitochondrial sheath.

The most modified spermatozoa of all are those of selachians and chimaeras, which are much hardier than primitive type spermatozoa. The head is elongated and has a corkscrew shape. The acrosome is spear-shaped. The mitochondrial sheath of the middle piece is highly developed, twisting in a helix around the axial filament for a considerable part of its length and is similar in structure to that of gastropods and mammalian spermatozoa. The presence of a pair of thick accessory fibers in the tail of selachian and chimaeran spermatozoa resembles the structure in gastropods, insects, birds and mammals (which have 9 accessory fibers arranged in a ring around the outer fibrils of the basic 9 + 2 complex). Apparently, progressive development of the mitochondrial sheath and the axial complex of the spermatozoon flagellum is related in all these cases with internal fertilization and the special conditions of gametic association in the genital ducts.

2.3. SPERM

2.3.1. Sperm of Fishes with External Fertilization

In all fishes which deposit sperm in the water, the milt is a suspension of individual spermatozoa in seminal fluid. The sperm is white or yellowish, and resembles milk.

In this group of fishes, the sperm is a testical secretion to which discharges of the accessory glands are generally not added. Perciformes (suborders Gobioidei and Blennioidei) are exceptional in having accessory glands in the form of unpaired seminal vesicles, which are not homologous with organs of the same name in higher vertebrates.

Many Gobioidei have a seminal vesicle, which represents a specialized part of the testis (Rathke, 1824, 1836; Hyrtl, 1850; Eggert, 1931; Coujard, 1941b; Weisel, 1949). It develops at the point of the anlage where the testes join, does not serve to store sperm and produces a viscous protein-rich secretion (Young and Fox, 1937) which mixes with the sperm on ejection. It was suggested that this secretion prolongs the life of spermatozoa in the spawn. In experiments with Gobius panizae (Eggert, 1931), the addition of the seminal vesicle secretion to sperm in seawater prolonged the active motility of the spermatozoa from 30—50 to 50—60 minutes. However, in the case of Gillichthys mirabilis (Young and Fox, 1937; Weisel, 1948, 1949), no effect of seminal vesicle secretion on the duration of the spermatozoan movement was established.

The seminal vesicle of Blennidae is an outgrowth of the efferent duct of the testis (Eggert, 1931); it serves to store the sperm and, in addition, its epithelium shows signs of secretory activity.

Apart from the seminal vesicle, still another accessory gland is found in some Gobiidae and Blenniidae. Its tissues are located at the testical surface and also in islet form between the seminiferous tubules; the cells contain many lipid inclusions. Some investigators (Champy and Gley, 1922; Coujard, 1941a, b) regard this as an exocrine gland and describe excretion of a lipid-rich substance during ejection of the sperm. Another viewpoint (see Stanley et al., 1965) holds that this is an endocrine gland producing steroids and is a homolog of testical interstitial tissue in higher vertebrates.

In Astyanax mexicanus, the role of accessory gland is fulfilled by the glandular epithelium of the efferent duct of the testis, which discharges a lipid-rich secretion into the seminal fluid (Rasquin and Hafter, 1951).

The function of these glandular formations is still unclear. Considering the breeding habits of Gobiidae and Blenniidae, it has been suggested that the glandular secretion cements the ground area which the male prepares for protecting the eggs, or increases the viscosity of the filaments which attach the eggs to the nest, etc.

The seminal fluid contains little organic matter in fishes lacking accessory glands. In salmonids, in particular, it is similar in composition to physiological solution. The specific weight of this fluid in different salmonids is 1.008—1.010. The pH varies in rainbow trout from 7.0 in fluid sperm to 7.8 in thick sperm of good quality (Scheuring, 1928) and in Coregonus lavaretus from 7.5 to 9.5 (Medem et al., 1949).

The seminal fluid of Salmo salar (Miescher, 1896) is transparent and colorless. It contains 0.13—0.19% organic matter (with traces of protein) and 0.65—0.75% inorganic salts. The seminal fluid of Salmo fontinalis (Felix et al., 1956) is transparent with a yellowish tinge. It contains 1.76% N and 0.43% P; the N/P ratio is 4.19. The seminal fluid of rainbow trout (Schlenk and Kahmann, 1938) contains (in milliequivalents per cc substrate): 0.1334 Na, 0.0202 K, 0.1302 Cl, 0.0099 SO_4 and 0.0012 PO_4; the pH is 7.3. Thus, the seminal fluid of trout contains 6.6 times as much sodium as potassium.

The total salt content may vary considerably: in trout (Scheuring, 1928) the freezing point of the seminal fluid decreases from -0.056°C for fluid sperm to -0.38°C for good-quality sperm. Thus, the salt content in sperm of trout is lower than in sperm of warm-blooded animals whose freezing point is between -0.55 and -0.61°C. Furthermore, the sperm of salmonids is hypotonic compared to their blood (see Table 15).

Each cc of sperm contains billions of male gametes. In addition to spermatozoa, the sperm of some Cottidae also contains special bodies (Hann, 1927, 1930) of not quite regular spherical shape larger than the spermatozoa (their diameter is about 10μ). These bodies have been found in the sperm of representatives of 4 genera of Cottidae, both freshwater and marine. In one case, Cottus bairdii bairdii, it was shown that the sperm of fishes from certain habitats — the southeastern part of Michigan state — contains these bodies, but not sperm of fishes from waters in the western and northern parts of this state. The bodies appear during spermatogenesis, apparently through the union of several spermatids. This is why they were called spermatid masses. The link between their formation and the geographical locality indicates that certain external factors, possibly temperature, influence their formation.

Similar formations were found also in the sperm of two species of Baikal sculpins, Cottus kneri and Cottocomephorus grewingki (A.F. Turdakov, personal communication).

The amount of sperm discharged by male fishes at a single time varies considerably, from hundredths and tenths of a cc in some teleosts to many thousand cc in the basking shark (Table 20). The volume of the ejaculate is correlated to numerous factors: size of the male, duration of its participation in spawning and number of ejaculations, number of eggs spawned at a single time by the female, the relative number of spawning males and

TABLE 20. Volume of sperm ejected by a male at a single time

Family	Species	Volume, cc			Author
		minimum	maximum	average	
Lamnidae	Cetorhinus maximus			~18,000	Harrison Metthews, 1950
Acipenseridae	Huso dauricus		800–1,000		Svirskii, 1966
	Acipenser güldenstädti colchicus	25	500	166,8	Ginzburg, unpublished
	Acipenser stellatus	25	200	110,8	G. M. Ignat'eva and E. V. Chulitskaya, unpublished
Salmonidae	Oncorhynchus keta infsp. autumnalis	3.6	17.9	9.2	Smirnov, 1963a
	Oncorhynchus gorbuscha	0.5	21.7	6.5	
	Oncorhynchus masu	1.4	52.0	12.8	
	Oncorhynchus nerka	0.3	28.0	9.9	
	Oncorhynchus kisutsch	0.5	90.5	19.9	
	Salmo gairdneri			3.5	Nursall and Hasler, 1952
	Salmo trutta				
	Coregonus lavaretus			1	Medem et al., 1949
Esocidae	Esox lucius			<1	Lindroth, 1946
Cyprinidae	Diptychus dybowskii	2	10	3.5	Turdakov, 1962
	Schizothorax pseudaksaiensis issykkuli	2	5	3	
	Leuciscus bergi	0.03	0.5	0.2	
	Rutilus rutilus caspicus	2	4		Tereshchenko, 1913
	Cyprinus carpio	2.36	3.44	2.90	Clemens and Grant, 1965

females, conditions of spawning (in small groups or in large schools as well as current velocity at spawning grounds since spermatozoa are more quickly dispersed in a rapid current).

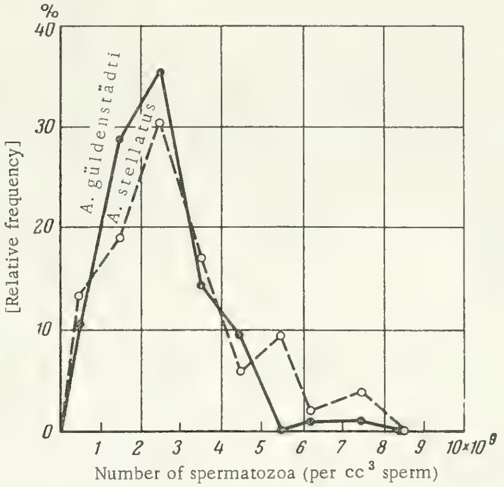

FIGURE 33. Distribution of spermatozoan concentrations in sperm of Acipenser güldenstädti and A. stellatus.

Sperm samples were checked from 105 A. güldenstädti and 53 A. stellatus. The y-axis indicates the relative frequency of occurrence of males with a given spermatozoan concentration in the ejaculate.

The relation between the amount of sperm discharged and these factors may be illustrated by a few examples. Among Pacific salmons of the genus Oncorhynchus (Smirnov, 1963a), the sockeye spawns in a slow current, but the silver salmon and masu spawn in a rapid current; accordingly the sockeye discharges less milt at a time than the silver salmon or masu (Table 20). In pink salmon, which spawns in quite a rapid current, the small volume of ejaculate is compensated by the large number of males participating (5–8 males per female).

The Issyk-kul cyprinid Leuciscus bergi, Diptychus dybowskii and Schizothorax pseudaksaiensis issykkuli differ in their spawning ecology. Spawning Leuciscus bergi gather in large schools in which the ratio of ripe females to males is approximately 1:8. Since Diptychus dybowskii and Schizothorax pseudaksaiensis issykkuli do not congregate (Turdakov, 1962), the amount of sperm discharged at a time by the two species is 15 times greater than that discharged by Leuciscus bergi (Table 20).

There is considerable variation not only in the volume of the ejaculate but in the spermatozoan concentration per unit volume (Table 21). In sturgeons, which discharge a large volume of sperm at a single time, the concentration of spermatozoa per unit volume is one-tenth to one-fifth that

TABLE 21. Number of spermatozoa per cc of ejaculate

Family	Species	Number of sperm samples	Number of spermatozoa, 10^9			Author
			minimum	maximum	average	
Acipenseridae	Huso huso	18	0.58	6.40	2.51	Ginzburg, unpublished
	Acipenser ruthenus		0.59	2.41		Persov, 1953a
	Acipenser güldenstädti	3	1.07	3.16		Persov, 1953a
	Acipenser güldenstädti colchicus	82	0.14	7.55	2.56	Ginzburg, unpublished
	Acipender güldenstädti persicus	9	0.6	1.5		Persov, 1947b
	Acipenser stellatus	19	0.90	10.37	3.19	Persov, 1947b
		53	0.12	7.30	2.89	Ginzburg, unpublished
Clupeidae	Caspialosa kessleri	1			73.2	Ginzburg, unpublished
	Clupea harengus pallasi		80	100		Yanagimachi, 1957b
Salmonidae	Oncorhynchus kela infsp. autumnalis	5	5.60	32.40	24.12	Smirnov, 1963a
	Oncorhynchus gorbuscha	36	8.32	29.04	17.94	
	Oncorhynchus masu	42	16.80	36.80	21.23	
	Oncorhynchus nerka	22	4.02	18.22	10.56	
	Oncorhynchus kisutsch	32	6.34	32.75	17.34	
	Salmo trutta m. lacustris	26	9.15	26.06	16.33	Ginzburg, unpublished
	Salmo irideus		12.9	28.1	20.4	Clemens and Grant, 1965
Esocidae	Esox lucius		20.3	23.0		Lindroth, 1946
		1			14.02	Persov, 1953a
Cyprinidae	Leuciscus idus	1			16.07	Persov, 1953a

TABLE 21 (Continued)

Family	Species	Number of sperm samples	Number of spermatozoa, 10^9			Author
			minimum	maximum	average	
Cyprinidae	Rutilus rutilus				11.1	Musselius, 1951
	Rutilus rutilus caspicus		7.6	12.8	9.36	Tereshchenko, 1913
	Tinca tinca				4.9	Musselius, 1951
	Abramis brama		10.0	16.0		Tereshchenko, 1917
					19.1	Musselius, 1951
		1			12.10	Persov, 1953a
	Leuciscus bergi		3.12	14.16	7.67	Turdakov, 1963
	Schizothorax pseudaksaiensis issykkuli		10.81	21.92	15.06	Turdakov, 1962
	Diptychus dybowskii		12.80	27.60	20.78	Turdakov, 1962
	Carassius carassius				18.7	Musselius, 1951
		1			24.0	Persov, 1953a
	Cyprinus carpio				26–28	Musselius, 1951
			23.8	25.6	24.7	Clemens and Grant, 1965
Cobitidae	Misgurnus fossilis		2.48	4.79		Persov, 1953a
Percidae	Perca fluviatilis				35.6	Musselius, 1951
		1			52.0	Ginzburg, unpublished
	Lucioperca lucioperca	2	14.07	16.49		Persov, 1953a

of most salmonids and cyprinids. Accordingly, good-quality sturgeon sperm outwardly resembles unskimmed milk, whereas trout sperm which contains more than 20 billion spermatozoa per cc has the consistency of dense cream. The concentration of spermatozoa in milt of salmonids and cyprinids is much higher than in mammalian ejaculate whose average numbers of spermatozoa per cc vary between 100 million in the boar and 120 million in the stallion to 3 billion in the ram and $3\frac{1}{2}$ billion in the billy goat (Bishop, 1961).

Figure 33 shows the distribution of spermatozoan concentrations in sperm of Acipenser güldenstädti and A. stellatus. The overwhelming majority of males eject sperm containing from 1 to 4–5 billion spermatozoa per cc; higher concentration are exceptional. The concentration varies not only in different males of the same species but even in different ejaculates obtained from the same male.

2.3.2. Sperm of Fishes with Internal Fertilization

The composition and properties of sperm of fishes in which fertilization occurs within the female have hardly been studied. The scant data available in the literature indicate that the spermatozoa are not dispersed individually in sperm of these fishes, but form large aggregates without membranes or spermatophores with dense outer membranes. Such aggregation of spermatozoa reduces losses in the passage to the female genital ducts.

The formation of true spermatophores in selachians and chimaeras with discharges of Leydig's duct (the specialized anterior part of the mesonephric duct) and of the expanded lower section of the efferent duct (Wolffian duct) has been described.

In the basking shark (Cetorhinus maximus) formation of spermatophores occurs in the ampulla ductus deferentis, the expanded lower section of the symmetrical Wolffian ducts (Harrison Matthews, 1950). Each ampulla reaches 2 m in length and contains about 25 liters of sperm. The spermatozoa mix with the Leydig's duct secretion in the upper, spiral part of the Wolffian duct and form small clumps in the anterior part of the ampulla, where they enter pouches formed by folds of the mucous membrane. The ciliary action of the epithelial lining of the pouches rotates the clumps, while a gelatinous glandular secretion of mucous cells is deposited in concentric layers around the spermatozoan clumps. On congealing, this secretion forms the dense spermatophoran membrane. The spermatophores formed are irregular spherical bodies with diameters of 2–3 cm or more. The central mass of spermatozoa (up to 1 cm in diameter) is surrounded by a thick layer of semitransparent hyaline material. This apparently protein material shows no reaction to mucin. On entering the female genital duct, the dense hyaline substance of the spermatophore softens and becomes gelatinous, which allows for release of the spermatozoa.

A discharge of the accessory glandular organ (siphon of the clasper) is added to shark sperm on ejection. It has been established that the viscous yellowish secretion of this organ in Squalus acanthias contains an unusually high concentration of serotonin (see Mann, 1964).

In Chimaera monstrosa, free spermatozoa are found in the upper part of the winding vas deferens; these unite into spermatophores where Leydig's duct enters the vas deferens (Parker and Burland, 1909). These spermatophores are small ovate bodies covered by a dense membrane and filled with a gelatinous substance containing clusters of spermatozoa (Parker and Haswell, 1938).

In another chimaera, Hydrolagus (Chimaera) colliei, spermatophores apparently do not form. The anterior part of the ampulla ductis deferentis is filled with spermatozoa in clusters, the middle part contains a bluish green gelatinous secretion and the posterior part contains a light-colored fluid (Stanley, 1963). On ejection of the semen, it may be assumed that the gelatinous secretion envelops the spermatozoan clusters and helps prevent loss of sperm on the passage to the female genital duct, as is the case of spermatophores.

Among teleosts, true spermatophores were described (Kulkarni, 1940) only in the case of one species, Horaichthys setnai, which is the sole representative of the family Horaichthyidae (order Cyprinodontiformes). These are small transparent fishes inhabiting tidal waters of the west coast of the Indian subcontinent. Male H. setnai, 19–23 mm long, have a complex gonopodium (Figure 34, a). Each male produces 250–280 elongated, pear-shaped spermatophores measuring 0.1 × 0.6 mm (Figure 34, c). During courtship, the male approaches the female with lightning speed, striking her with the gonopodium in the region of the genital pore. At this instant the spermatophores are released and attach to the area bordering the genital pore (Figure 34, b) by means of the several rows of sharp denticles of its spear-shaped end. A swelling soon appears at the base of the armed end and fills with active motile spermatozoa, which pass outside after the hyaline wall of the spermatophore is ruptured (Figure 34, d). The spermatozoa penetrate into the oviduct and collect in folds of its wall. Here the spermatozoa retain fertility for a long time. Fourteen days after females were separated from males fertilized eggs were spawned. Since the blastodisc in these eggs was hardly formed, it may be assumed that fertilization occurred immediately before spawning.

In the livebearers (family Poeciliidae) among cyprinodonts, spermatozoa unite into aggregates, which lack an external membrane and thus cannot be called spermatophores. The spermatozoa are arranged radially in such aggregates with the heads outermost and joined by a special cementing substance. Such formations were first described for Poeciliidae by Philippi (1908), who called them "spermozeugma" which is a term proposed by Ballowitz for similar aggregates found in insects.

Spermozeugmas of cyprinodonts are milk-white bodies suspended in seminal fluid, In Cnesterodon decemmaculatus, Poecilia (Lebistes) reticulata, Phalloceros caudimaculatus and Heterandria formosa, spermozeugma are ellipsoidal and approximately twice as long as wide (see Table 22). In 7 species of the genus Xiphophorus (including those earlier referred to the genus Platypoecilus) the spermozeugmas are round, oval or ovate, and in Poecilia (Limia) melanogaster they are drop-shaped (Philippi, 1908; Zander, 1961). Spermozeugmas of the guppy often have a small constriction in the middle, resembling peanuts in shape; they usually break up by dividing into half at this constriction.

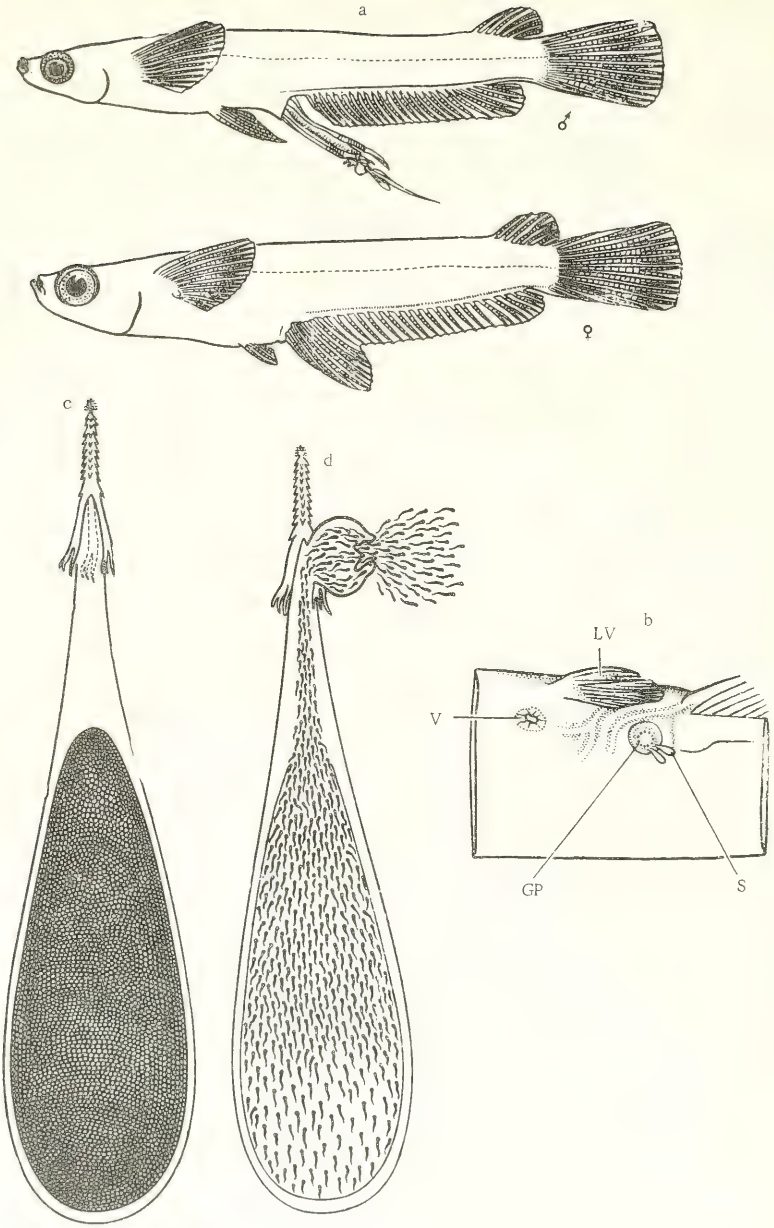

FIGURE 34. Spermatophores of Horaichthys setnai:

a — external view of male and female; b — region of female genital pore, two spermatophores are shown attached; c — form of spermatophores; d — release of spermatozoa from spermatophore (after Kulkarni, 1940). V — anal vent; LV — left ventral fin (right ventral fin usually absent); GP — genital pore; S — spermatophore.

The number of spermozeugmas produced by males of various species of platys seems to vary considerably. In the case of artificial production of sperm it was possible to collect at a single time up to 3,000 spermozeugmas from Xiphophorus helleri, 2,000 from Poecilia melanogaster, 350 from guppy and 20 from Heterandria formosa. The number of spermatozoa also varies according to the size of the spermozeugma: a single guppy spermozeugma contains 14,000—17,000 spermatozoa, that of the swordtail 4,000—5,500 (Zander, 1961).

TABLE 22. Size (in μ) of spermozeugmas in Poeciliidae

Species	Average	Minimum	Maximum	Author
Cnesterodon (Glaridichthys) decemmaculatus	107 × 220			Philippi, 1908
Poecilia (Lebistes) reticulata	84 × 190	70 × 140	120 × 260	Ginzburg, unpublished
Phalloceros caudimaculatus*	73 × 122			Philippi, 1908
Xiphophorus helleri	64 × 79	60 × 60	90 × 115	Ginzburg, unpublished

* Philippi identified this species incorrectly as Glaridichthys januarius (see Henn, 1916).

The spermozeugmas are incapable of active movements and insemination occurs through actual mating and direct transfer of the sperm by means of the gonopodium (rather than "shooting" and adhesion of the spermozeugmas to the female sexual papillae, as earlier supposed; see Petzold, 1967).

The spermozeugmas of Phalloceros caudimaculatus and Cnesterodon decemmaculatus remain unchanged in physiological solution for three days, but in the presence of ovarian fluid the cementing substance dissolves within several minutes and spermatozoa are seen to move about energetically (Philippi, 1908). On the other hand, spermozeugmas of guppy and swordtail clump together in Ringer's solution and break up into individual spermatozoa quite soon (Ginzburg, unpublished data).

After the spermozeugma breaks up in the oviduct, the free spermatozoa reach the ovary and fertilize the oocytes in the follicles. A large number of spermatozoa accumulate in the folds of the mucous oviduct where they remain fertile for a very long time. In guppy, a single insemination suffices the female for 5—11 egg batches (see Petzold, 1967); cases of females isolated from males, which continued to produce young for 12—14 months, have been recorded (Natali and Natali, 1931).

2.4. MOTILITY AND FERTILITY OF SPERMATOZOA

2.4.1. Sperm Motility in Water

The spermatozoa are immotile in seminal fluid. On entering water they instantly become activated and swim about energetically. The movement of the spermatozoon is only rarely rectilinear; usually it swims in a curved, often spiral direction (Adolphi, 1906; Schlenk and Kahmann, 1935).

When teleost spermatozoa encounter a hard surface in their forward movement, a peculiar phenomenon is observed which was described earlier for spermatozoa of some insects (Dewitz, 1886) and sea urchins (Gray, 1955). The spermatozoa begin to move in a curve in a direction constant and characteristic for each species. In some species most spermatozoa execute a clockwise curve, in other species counterclockwise (Gibbons, 1962). There are only brief data for fishes, but it was indicated for the sea urchin Strongylocentrotus purpuratus that 99.9% of the spermatozoa curve counterclockwise; the diameters of the curves are more or less constant (about 50μ). Analysis of the movement of sea urchin spermatozoa (Gray, 1955; Gibbons, 1962) showed that it usually consists of three components: forward movement, deviation to one side and rotation around its axis. The proximity of a hard surface causes rotation, so that in combination with the other two components movement in a closed circle results in a direction determined by the deviation of rectilinear movement. This helps explain why fish spermatozoa often move in a spiral in the water mass.

Unlike mammalian spermatozoa, those of fishes do not swim against the flow of the fluid (Adolphi, 1906).

There are considerable differences in the speed of spermatozoa of different fishes. Among fishes studied, maximum rates were observed in trout, whitefish, osman and marinka (164—330μ/sec). These rates resemble those of echinoderm and mammalian spermatozoa (Table 23).

TABLE 23. Rate of forward movement of spermatozoa

Class	Species	Temperature, °C	Maximum rate, μ/sec	Author
Bivalvia	Ostrea gigas	28	50	Fujita, 1930
Echinoidea	Echinus	18	120	Bishop, 1962
	Psammechinus miliaris	18	190	Gray, 1955
Pisces	Huso huso	16	100	Ginzburg, unpublished
	Salmo trutta m. fario	12.5	164	Schlenk and Kahmann, 1937
	Coregonus lavaretus maraena	11	180	Adolphi, 1906
	Esox lucius	21	100	
	Abramis brama	19	50	
	Leuciscus idus	19	33	
	Leuciscus bergi		105	Turdakov, 1962
	Diptychus dybowskii		220	
	Schizothorax pseudaksaiensis issykkuli		300	
Mammalia	Equus caballus	37	87	Bishop, 1962
	Ovis aries	39	250	
	Bos taurus	38	352	
	Homo sapiens	37	100	

At first the energetic forward movement of the spermatozoa gradually decelerates, then fluctuates, and finally ceases entirely.

The rate of movement of trout spermatozoa decreases very rapidly (this was traced with great accuracy with filming technique; Schlenk and Kahmann, 1935, 1937). At temperatures of 12.5°C and 16°C, the forward motion of spermatozoa of Salmo trutta m. fario for 4 seconds after their activation by water is 160—164μ/sec. After 8 seconds it slows to 85—91μ/sec, after 16 seconds to 24—33μ/sec and after 26 seconds to 2—5μ/sec; forward motion ceases within 29 seconds after activation of the sperm by water.

In Huso huso, the deceleration is very slow (Ginzburg, 1959, unpublished data; Figure 35); this was determined in microscopic observations of spermatozoa by recording (with a timer) the time required by a single spermatozoon to pass through a known linear distance. The maximum speed developed by Huso huso spermatozoa in the first seconds after activation by water, 100μ/sec at 16.2°C, was somewhat lower than in trout. Two minutes after water is added, many spermatozoa still move at a speed of 72μ/sec, after 5 minutes the speed was reduced to 16μ/sec, after 7—13 minutes only a few spermatozoa moved at no more than 10μ/sec, and all movement ceased within 14—15 minutes.

In some fish species, the deceleration of the spermatozoa in the suspension does not take place synchronously, and there appeared to be long- and short-lived spermatozoa; this was first noted during a study of overripe trout sperm and in experiments on the effect of certain electrolytes on spermatozoa (Scheuring, 1925). In spermatozoa of Salmo trutta m. lacustris, this difference among spermatozoa is constant and independent of the quality of the sperm and is observed in dilution with cavity fluid or Ringer's solution (Ginzburg, 1963a, b). In most spermatozoa forward movement continues for about 1 minute, but certain individual spermatozoa retain their capacity for forward movement for 20—46 minutes.

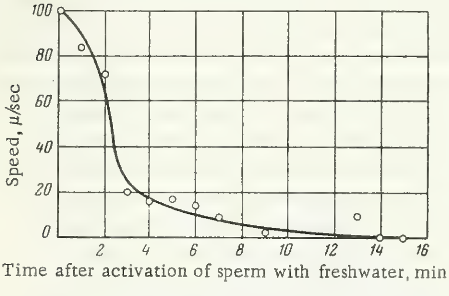

FIGURE 35. Dynamics of forward speed of Huso huso spermatozoa in water (temperature, 16.2°C)

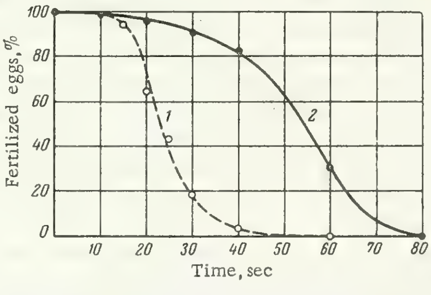

FIGURE 36. Decrease in fertility of lake trout spermatozoa in various dilutions of sperm (temperature, 2.9°C).

Sperm diluted by water, 1:50 (1) and 1:500 (2).

This was also observed in sperm of Caspialosa volgensis (Stroganov, 1938a) when diluted with water. After 40 seconds, 50% of the spermatozoa were still moving and only 10% after 66 seconds, but individual spermatozoa remained motile for up to 30—40 minutes.

Great variations in duration of the active period of different spermatozoa were determined for Acipenser güldenstädti colchicus (Ginzburg, unpublished). Observations were carried out on the sperm of 20 males at temperatures of 15–17°C. A small amount of "dry" sperm was placed in a drop of water on a slide, thoroughly mixed and covered with a cover slip. The movements of the spermatozoa were followed under a microscope (mag. 120 ×). The energetic forward movement of most spermatozoa begins just after the sperm is diluted with water, but a small percentage (varying in the sperm of different males) is usually not activated. There is no appreciable decrease in the percentage of motile spermatozoa in the first minute; thereafter some begin to waver and, generally, after 3 minutes less than half the spermatozoa still swim. After 5–10 minutes only isolated long-lived spermatozoa were moving, but these retain the capacity for movement considerably longer; in the sperm of some males these spermatozoa moved about up to 20–30 minutes, in others up to 60 minutes or even longer, and in one batch out of the 20 males isolated spermatozoa moved for more than 3 hours.

The reasons for the variations in the period of active movement in spermatozoa from a single batch are not clear, but differences in the age of these spermatozoa and, correspondingly, their degree of maturity must play a decisive role.

The speed of spermatozoan movement (as well as the rate of biochemical reactions which make movement possible) depends on temperature. Since the energetic resources of the spermatozoon are limited, the increased speed caused by a temperature increase shortens the period of motility. In lake trout (Schlenk and Kahmann, 1937), forward movement ceases completely within 29 sec at 12.5–16°C and within 56 sec at 2.25°C. The total distance covered by the spermatozoa scarcely changes – 2.3 mm on the average, varying from 2.0 mm at highest temperatures to 2.6 mm at 2.25°C and 6.0°C.

The period of motility may also be affected by other factors, particularly by the concentration of spermatozoa in the suspension.

Thus, in an experiment with lake trout (Ginzburg, unpublished; Figure 36), sperm was diluted by water to various proportions (1:50 and 1:500), and then spawn was inseminated after different time periods. In the more concentrated sperm suspension, the decrease in fertility began much earlier than in the more dilute suspension; consequently, the period of active movement of spermatozoa in the denser suspension was much shorter (see p. 139). The "dilution effect" on increased activity of spermatozoa in suspenion established long ago for sea urching spermatozoa is apparently determined simultaneously by several factors which change directly with changes in spermatozoan concentration: oxygen and carbon dioxide partial pressures and pH of the medium. (Gray, 1928a; Rothschild, 1948b; Mohri and Vasumasu, 1963).

The total motile period of the spermatozoa differs greatly in various fishes (Table 24). It is noteworthy that this period is much longer in fishes spawning in brackish water and seawater than in most fishes spawning in freshwater. Considerable variations can be observed even within each group; in particular cases the physiological bases of these variations are already clear. For example, in Issyk-kul cyprinids (Diptychus dybowskii, Schizothorax pseudaksaiensis issykkuli, Leuciscus bergi) which spawn at 5.8 ‰ salinity, the period of spermatozoan motility varies considerably; in the former two species it lasts 20–25 minutes, in the latter species 10 hours (Turdakov, 1962). This feature of the spermatozoa of Leuciscus bergi is related to a peculiar periodicity in the forward motion of the spermatozoa, which periodically slow and cease moving, allowing for conservation of energetic resources for a longer period. This is carried to an extreme in spermatozoa of Clupea harengus pallasi, which live for 4–5 days in seawater (Galkina, 1957); insemination of eggs 48 hours after dilution in water leads to fertilization of 62% of the eggs (Yanagimachi, 1957b). Live Pacific herring spermatozoa display very slight motility; most perform only with fluctuating movements (of amplitudes approximately equal to the width of the sperm head), while only a few move forward from time to time during several days (Galkina, 1957). Energetic movement is acquired only in the immediate vicinity of the egg (Yanagimachi, 1957c).

The spermatozoa of most fishes spawning in freshwater remain motile in water for no longer than 2–3 minutes.

Early works on salmonids indicated a longer period of motility of spermatozoa in water: 7–8 minutes in brook trout (Coste, see Henneguy, 1888) and up to 5 minutes in chinook salmon (Rutter, 1904). These figures are too high and result from insufficient mixing of the sperm with water; this error was proven experimentally in trout (Henneguy, 1888). Apparently, the same error occurred in experiments on chinook salmon, since in a mixture of sperm with water the spermatozoa remain motile and are fertile (although to a low degree) for 5–7 minutes; however, in a drop of suspension under a microscope their movement, which is very active for the first 30 seconds, ceases completely within 65 seconds (Rutter, 1904, p. 73).

Contrary to results obtained in numerous investigations on various species of salmonids and reported in Table 24 A, the data indicate that Oncorhynchus nerka spermatozoa retain fertility for longer periods (Foerster, 1965); fertilization of a certain percentage of eggs was possible at 2–8°C 1–8 hours after dilution of the sperm. The loss of spermatozoan fertility in the different experiments occurred after various times (sometimes within several minutes) and the percent of fertilized eggs in consecutively inseminated samples in a single experiment showed very great irregularities. Foerster noted that sperm of Oncorhynchus nerka was thoroughly mixed with water, thus the reasons for such divergent and inconstant results are not clear.

The period of spermatozoan activity in sturgeons and loach is much longer than in most fishes spawning in freshwater and may last for several or even many hours.

The duration of the spermatozoan activity is correlated to spawning conditions. It is shorter in species spawning in cold water (salmon, trout, burbot) than in fishes spawning in spring (pike, tench, crucian carp) if calculated for a uniform temperature; spermatozoa of pike, on the other hand, swim for a shorter period than those of tench and crucian carp which spawn at later dates. However, since the swimming period of spermatozoa is reduced at elevated temperatures, the absolute duration of activity in these species is similar at spawning temperatures (Lindroth, 1947). Moreover, spermatozoa of fishes spawning in a rapid current lose motility more rapidly than spermatozoa of fishes spawning in calm water (Haempel, 1913; Nikol'skii, 1963). Spermatozoa of the first group need not retain motility

TABLE 24. Duration of spermatozoan movement in water (at salinities corresponding to spawning conditions)

Family	Species	Temperature, °C	Movement		Retention of fertility	Author
			total duration	duration of energetic movement		
A. Fishes spawning in freshwater						
Acipenseridae	Huso huso	16.2		13 min		Ginzburg, unpublished
	Acipenser güldenstädti		5–9.5 min	3.5–5 min		Drabkina, 1961
	Acipenser güldenstädti colchicus	15–17	5 min–3 hr 15 min		20 min–12 hr	Ginzburg, unpublished
	Acipenser stellatus		40–15 min			Borodin, 1898
	Acipenser ruthenus	18.6–20.2			3 min–2.5 hr	Ginzburg, unpublished
		12–13		5–6 hr		Shmidtov, 1936
Clupeidae	Caspialosa volgensis	22.5	Majority <66 sec	A few 30–40 min		Stroganov, 1938a
Salmonidae	Oncorhynchus gorbuscha	10–11	70–170 sec	30–55 sec		Smirnov, 1954
		10			50–150 sec	Smirnov, 1963
	Oncorhynchus tschawytscha		65 sec		60 sec	Rutter, 1904
	Salmo salar		45 sec			Haempel, 1913
		5		85 sec		Lindroth, 1947
		23		27 sec		
		3.4			>60 sec	Novikov, 1953
	Salmo salar m. sebago			70–110 sec		Privol'nev, 1941
	Salmo trutta m. lacustris	3	203 sec	63 sec	40–60 sec	Ginzburg, 1963a, b; unpublished data
		9	125 sec	40 sec		
		10–12.5			30 sec	

Note. All data from this work are taken from graphs.

TABLE 24 (Continued)

Family	Species	Temperature, °C	Movement		Retention of fertility	Author
			total duration	duration of energetic movement		
	Salmo trutta m. fario		30 sec		15 sec	Henneguy, 1877, 1888
			23 sec			Haempel, 1913
			90–150 sec			Gray, 1920
			87–97 sec	30–40 sec		Scheuring, 1928
		11–13	85 sec	36 sec		Medem et al., 1949
		2.5		60 sec		Schlenk and Kahmann, 1937
		9		40 sec		
		16		29 sec		
	Salmo irideus		40 sec			Haempel, 1913
			20–40 sec			Schlenk, 1933
		13	60–105 sec	20–45 sec	45 sec	Dorier, 1951b
	Salvelinus fontinalis		65 sec		60 sec	Werner, 1934
			90–210 sec			Allison, 1961
	Salmo hucho		45 sec			Haempel, 1913
	Coregonus lavaretus		50–60 sec			Surbeck, 1916
		11–13	118–131 sec	66–89 sec		Medem et al., 1949
	Coregonus muksun			45–60 sec		Bakhareva, 1934
Thymallidae	Thymallus thymallus	11–15	48–54 sec	28–34 sec		Roth et al., 1950
Esocidae	Esox lucius		3–4 min			Haempel, 1913
				20–30 sec		Heuschmann, 1940
		3.5		150 sec		Lindroth, 1947
		5		120 sec		Lindroth, 1946
		15		60 sec		
		8.5			120 sec	
		13.5			75 sec	
		11–15	105–179 sec	60–88 sec		Roth et al., 1950

TABLE 24 (Continued)

Family	Species	Temperature, °C	Movement		Retention of fertility	Author
			total duration	duration of energetic movement		
Cyprinidae	Rutilus rutilus		120–240 sec			Musselius, 1951
	Rutilus rutilus caspicus		138 sec			Ivlev, 1940
		15.5	111 sec	81 sec		Tanasiichuk and Vonokov, 1956
	Scardinius erythrophthalmus	7.6		128 sec		Lindroth, 1947
		19		58 sec		
	Tinca tinca	7.2		210 sec		
		19		90 sec		
			100–120 sec	55 sec		Elster and Mann, 1952
			11.5 min			Musselius, 1951
	Barbus barbus (= B. fluviatilis)		120 sec			Haempel, 1913
	Chalcalburnus chalcoides danubicus		1–2 min			Doroshev and Gorelov, 1964
	Abramis brama		104 sec			Ivlev, 1940
		7.2		150 sec		Lindroth, 1947
		19		75 sec		
		16.2	77 sec	53 sec		Tanasiichuk and Vonokov, 1956
	Abramis brama orientalis	20	10 min	8 min		Gosteeva, 1957
	Blicca bjoerkna (=Abramis blicca)	5		97 sec		Lindroth, 1947
		21		45 sec		
	Vimba vimba vimba caninata	15–17	40–60 sec		35 sec	Smirnova and Kuz'mina, 1966
		19–24	30–35 sec		15–30 sec	
	Carassius carassius	7.5		240 sec		Lindroth, 1947
		19		98 sec		
			60–138 sec			Musselius, 1951
	Carassius carassius auratus	18–21	3 min	30 sec		R. Suzuki, 1959b

TABLE 24 (Continued)

Family	Species	Temperature, °C	Movement		Retention of fertility	Author
			total duration	duration of energetic movement		
	Cyprinus carpio		5 min			Haempel, 1913
			87 sec			Ivlev, 1940
			90–180 sec	60 sec		Musselius, 1951
			100–120 sec	55 sec		Elster and Mann, 1952
			60 sec			Belyaev, 1957
		13–21	3 min	30 sec		R. Suzuki, 1959b
			7–8 min			Doroshev and Gorelov, 1964
	Sarcocheilichthys variegatus	18–21	6 min	3 min		R. Suzuki, 1959b
	Acheilognathus lanceolata	18–21	6 min	3 min		
	Acheilognathus tabira	18–21	6 min	3 min		
	Gnathopogon elongatus	18–21	1 min			
Cobitidae	Misgurnus fossilis		3 hr			Belyaev, 1957
	Misgurnus anquillicaudatus	18–21	1 min			R. Suzuki, 1959b
	Cobitis biwae	18–21	1 min			
Gadidae	Lota lota	5		70 sec		Lindroth, 1947
		20		18 sec		
Percidae	Lucioperca lucioperca		88 sec	46 sec		Tanasiichuk and Vonokov, 1955
	Perca fluviatilis	7		59 sec		Lindroth, 1947
		17		38 sec		
			60–138 sec			Musselius, 1951
	Acerina cernua	5		63 sec		Lindroth, 1947
		21		28 sec		

TABLE 24 (Continued)

Family	Species	Temperature, °C	Movement		Retention of fertility	Author
			total duration	duration of energetic movement		
B. Fishes spawning in brackish water						
Cyprinidae	Diptychus dybowskii			20 min		Turdakov, 1962
	Schizothorax pseudaksaiensis issykkuli			25 min		
	Leuciscus bergi	18–20		10 hr		Turdakov and Turdakov, 1959
	Chalcalburnus chalcoides aralensis		15 min			Doroshev and Gorelov, 1964
	Cyprinus carpio		26 min			
	Abramis brama orientalis	19.2	20 min	13 min		Gosteeva, 1957
C. Fishes spawning in seawater						
Clupeidae	Clupea harengus harengus				More than 7 days	Blaxter, 1955
	Clupea harengus pallasi				25 hr	Kryzhanovskii, 1955, 1956
		6–9			More than 2 days	Yanagimachi, 1957b
			Up to 4–5 days			Galkina, 1957
		4–5			24 hr	Galkina, 1963
Osmeridae	Mallotus villosus	4–5			24 hr	Galkina, 1963
Cyprinodontidae	Fundulus heteroclitus		8–14 min			Gee, 1916
Ammodytidae	Ammodytes tobianus				4 min	Thomopoulos, 1954
Gobiidae	Gobius panizzae			30–35 min		Eggert, 1931
	Gillichthys mirabilis	23–26	4–6 hr			Weisel, 1948
Cottidae	Cottus bubalis euphasen		20 min	14 min		Thomopoulos and Bauchot, 1957
Cyclopteridae	Cyclopterus lumpus	4–5			24 hr	Galkina, 1963
Gasterosteidae	Pungitius pungitius		20–90 min			Henneguy, 1888

for a long time, since the probability of encounters of the sperm and egg in a rapid current soon becomes negligible. This correlation is not universal. Although sturgeons spawn in a rapid current, their spermatozoa remain motile in water for a relatively long period.

The short period of activity of spermatozoa of most freshwater fishes matches the very rapid loss of fertility of the eggs. On the other hand, the prolonged motility of spermatozoa of sturgeons and fishes spawning in salt water matches the long retention of fertility in their eggs.

2.4.2. Spermatozoan Motility in Salt Solutions of Various Concentrations and in Cavity Fluid

As already mentioned, spermatozoa of fishes spawning in brackish and seawater swim much longer than those of most freshwater-spawning fishes.

If sperm of freshwater fishes is diluted experimentally with brackish water or physiological solution, the period of active movement of the spermatozoa is often lengthened (Table 25). This was observed clearly in experiments on sturgeon (Drabkina, 1961), salmon (Ellis and Jones, 1939), char (Werner, 1934), whitefish (Reighard, see B. I. Cherfas, 1950, p. 168), pike perch, roach, bream (Tanasiichuk and Vonokov, 1955, 1956; Gosteeva, 1957), Chalcalburnus chalcoides and carp (Doroshev and Gorelov, 1964).

The difference in duration of activity of the spermatozoa is particularly striking in the case of salmon. In freshwater they are motile for less than 2 minutes, whereas in diluted seawater movement continues for up to 180 minutes (maximally in a mixture with 15—20% seawater of 5—7‰ salinity).

In other investigations — on Acipenser stellatus (Borodin, 1898), Alosa volgensis (Stroganov, 1938a), trout (Scheuring, 1925), roach, bream, wild carp (Ivlev, 1940), pond carp and loach (Belyaev, 1957) — movement of the spermatozoa at higher salinities lasted as long as in freshwater or even shorter times. All these investigations (except for Ivlev) involved isolated observations. In experiments with series of consecutive salt concentrations (Ellis and Jones, 1939; Tanasiichuk and Vonokov, 1955, 1956; Drabkina, 1961), the duration of spermatozoan activity showed great variation. A substantial prolongation of this period is often observed only within a narrow concentration (about 5‰ for pike perch, roach and bream, 2‰ for sturgeon), whereas the difference is considerably less at other salt concentrations; when the concentration is increased, the period of spermatozoan movement decreases rapidly. The negative results in those works where a stimulating effect of increasing salt concentration was not observed were apparently due to the incomplete data; should different salinities be tried, prolongation of spermatozoan activity would probably be shown in these species.

It is not clear why Ivlev observed no prolongation of motion in spermatozoa of roach, bream and carp. Later, it was clearly demonstrated for two of these species that activity is prolonged at increased salinity (Tanasiichuk and Vonokov, 1956). The discrepancy may be due to the unfortunate criterion chosen by Ivlev; he calculated only "the time of termination of movement of approximately 90% of the spermatozoa" (1940, p. 472), whereas other investigators recorded the duration of forward motion and the total duration of spermatozoan movement.

When the salinity is increased above the optimal, not only is duration of movement of the spermatozoa shortened, but also the percent of active spermatozoa decreases; there is no activation at all above a certain concentration. In all freshwater fishes studied, spermatozoa are not activated at salinities higher than 13‰. In some activation becomes impossible even lower, e.g., for sturgeon spermatozoa at 8‰ salinity (Drabkina, 1961).

The opposite is observed in fishes spawning in seawater; their spermatozoa, which are motile in water of high salinity, are not activated by freshwater. Thus, active movement of Limanda schrenki spermatozoa is observed within the range of 15–35‰ salinity (these data were obtained by using seawater and a balanced salt solution at various dilutions). With a salinity of 4‰ or less, the spermatozoa remain immotile (K. Yamamoto, 1951b).

It is very interesting that in these marine fishes, as in freshwater fishes, the spermatozoa retain motility longest in water with a relatively low salinity and not in the natural medium. This was conclusively proven in Gillichthys (Weisel, 1948), and corroborated in Pacific herring (Yanagimachi, 1957a).

Spermatozoa of Gillichthys mirabilis (Weisel, 1948) are motile in a very wide salinity range from 6 to 68‰ (17–200% seawater). They are not activated at salinities lower than 6‰. The longest period of activity, an average of about 35 hours at 23–26°C, is observed at a salinity of about 8.5‰ (25% seawater). The activity is shorter in higher salinities: it lasts 4–6 hours in 100% seawater and about an hour in 200%. A decrease in temperature is accompanied by a sonsiderable increase in the swimming time of the spermatozoa; at 2–4°C, spermatozoa of Gillichthys in 20–25% seawater remain motile for 2 weeks.

The longest duration of spermatozoan movement in both freshwater and seawater fishes is observed at similar salinities which do not exceed 25% seawater (range from 2 to 8.5‰ salinity); the optimal salinity for sturgeon is 2‰ (Drabkina, 1961), 5‰ for pike perch, roach and bream (Tanasiichuk and Vonokov, 1955, 1956), 5–7‰ for salmon (Ellis and Jones, 1939) and about 8.5‰ for Gallichthys (Weisel, 1948).

The salt solutions in which spermatozoa remain active longest have a much lower osmotic pressure than the internal medium of these fishes. For example, in salmon the freezing point depression (Δ) of the optimal salinity (20% seawater) is -0.38°, for blood -0.61°; in the case of Gillichthys the Δ at the optimal salinity (25% seawater) is -0.48° and for blood approximately -0.84°. In nonelectrolyte solutions (sugars) spermatozoa of those same species swim longest in concentrations osmotically close to blood (the optimal sugar solution for salmon spermatozoa has a Δ = -0.48°, the optimal for those of Gillichthys is characterized by Δ = -0.96°). This difference with respect to seawater salts and sugars is apparently due to the fact that the surface membrane of the spermatozoon is less permeable to nonelectrolytes (Weisel, 1948).

What causes longer duration of spermatozoan movement in a medium of a certain osmotic pressure, particularly in water with a certain salinity

TABLE 25. Duration of fish spermatozoan movement in waters of various salinities and physiological solutions

Species	Freshwater	1–2 ‰	3–6 ‰	7–10 ‰	>10 ‰	Author
Acipenser güldenstädti	215–295 sec	185–445 sec	0–157 sec	0		Drabkina, 1961
Acipenser stellatus	40–45 min	30 min		Several seconds		Borodin, 1898
Caspialosa volgensis	60 sec		67 sec	57–73 sec		Stroganov, 1938a
Salmo salar	>1 min	10 min	60–180 min	30–120 min	15–20 min	Ellis and Jones, 1939
Trout	35 sec		29 sec	21 sec		Scheuring, 1925
	2 min			30 min		Huxley, 1930
Salvelinus (Salmo) fontinalis	>65 sec			480 sec		Werner, 1934
Whitefish	1 min			22 min		Reighard, see B. I. Cherfas, 1950
Rutilus rutilus	138 sec	133–139 sec	96–124 sec	<1–71 sec	<1 sec	Ivlev, 1940
	81 sec	135 sec	184 sec	107–132 sec	0	Tanasiichuk and Vonokov, 1956
Abramais brama	104 sec	101–102 sec	71–87 sec	26–66 sec	<1–10 sec	Ivlev, 1940
	53 sec	68 sec	83 sec	62–75 sec	0	Tanasiichuk and Vonokov, 1956
Cyprinus carpio	87 sec	81–83 sec	53–73 sec	<1–40 sec	<1	Ivlev, 1940
Misgurnus fossilis	3 hr		14–17 min			Belyaev, 1957
Lucioperca lucioperca	46 sec	61 sec	109 sec	0–93 sec	0	Tanasiichuk and Vonokov, 1955

range? One factor determining this may be a decrease in the energy expenditure for osmotic activity (Turdakov, 1962, 1963). This explanation, however, is unsatisfactory since spermatozoan activity lasts longest in hypotonic and not in isotonic solutions.

Another possible factor might be the protective effect of salt solutions on spermatozoa. In freshwater, a rapid change in spermatozoan structure takes place. The cytoplasmic sheath of the tail swells, divides into vacuoles and the tail becomes contorted (Hug et al., 1953); moreover, the motor apparatus of the spermatozoon is apparently irreparably damaged. The importance of the latter factor is confirmed in the case of Gillichthys (Weisel, 1948); spermatozoa placed in 10% seawater are immotile but can be activated by increasing the salinity, whereas spermatozoa kept for several minutes in fresh or distilled water cannot be activated.

In this connection, data should be given on the motility of spermatozoa in cavity fluid.

It has been shown for a whole series of fishes that their spermatozoa are activated in cavity fluid as in water, but preserve their motility considerably longer in cavity fluid. This was established for various species of trout (Scheuring, 1925; Hartmann et al., 1947b; Medem et al., 1949; Dorier, 1951a; Rucker et al., 1960; Ginzburg, 1963a, b), for Salmo salar (Ellis and Jones, 1939), Coregonus muksun (Bakhareva, 1934), Thymallus vulgaris (Roth et al., 1950) and Oncorhynchus kisutch (Rucker et al., 1960). All these fishes are salmonids. Spermatozoa are activated in cavity fluid more poorly than in water in Coregonus lavaretus among salmonids (Medem et al., 1949) and in the pike (Lindroth, 1946; Roth et al., 1950); not all the spermatozoa of these fishes acquire motility (not more than 80% in whitefish, only 30–50% in pike) and those that do, move less energetically. The duration of motility is prolonged, as in the species mentioned earlier. When the cavity fluid of whitefish and pike is diluted by an equal quantity of water, all the spermatozoa are immediately activated and the duration of movement increases considerably (pike spermatozoa swim 7–15 times longer in diluted cavity fluid than in water). The stimulating effect of cavity fluid is not species specific, a small proportion of grayling spermatozoa is activated in cavity fluid of pike, but in the diluted cavity fluid of pike all the grayling spermatozoa, like those of pike, acquire motility and swim longer.

In some fishes – Alosa volgensis (Stroganov, 1938a), Huso huso, Acipenser güldenstädti and A. stellatus (Ginzburg, unpublished data), loach (Belyaev, 1957; Ginzburg, unpublished data), Diptychus and Schizothorax (Turdakov, 1965), Rutilus frisii kutum (Smirnova, 1965) and Vimba vimba (Smirnova and Kuz'mina, 1966) – the spermatozoa are not activated at all in cavity fluid. However, the duration of activity increases 2–3 times when spermatozoa of Rutilus frisii kutum and Diptychus are placed in the diluted cavity fluid (Smirnova, 1965; Turdakov, 1965).

Since we have no data on the chemical composition of cavity fluid of different fish species, it is not clear why its effect on spermatozoa is so diverse. It is known only that the cavity fluid of species (e.g., sturgeons, loach and Schizothorax) which does not active spermatozoa has a considerable viscosity. When proteins in cavity fluid of loach and Schizothorax are coagulated by prolonged heating (Belyaev, 1957; Turdakov, 1965), the spermatozoa then become activated in the fluid. This suggests that proteins in the cavity fluid of these fishes play a role in immobilizing spermatozoa.

2.4.3. Correlation of Motility and Fertilizing Capacity of Spermatozoa; Individual Variations in Sperm Quality

An undoubted correlation exists between motility of spermatozoa and their capacity to fertilize eggs.

Special motion picture studies on trout spermatozoa (Schlenk and Kahmann, 1935) were employed to determine the speed, as well as fertilizing capacity, of spermatozoa kept for various periods in water. These experiments indicated that the percent of fertilization decreases as the speed of movement of the spermatozoa decreases; when movement ceases the capacity for fertilization is lost completely (Figure 37). However, a slight deviation was noted; a very small percentage of eggs continued to become fertilized for several seconds after the film showed immotile spermatozoa.

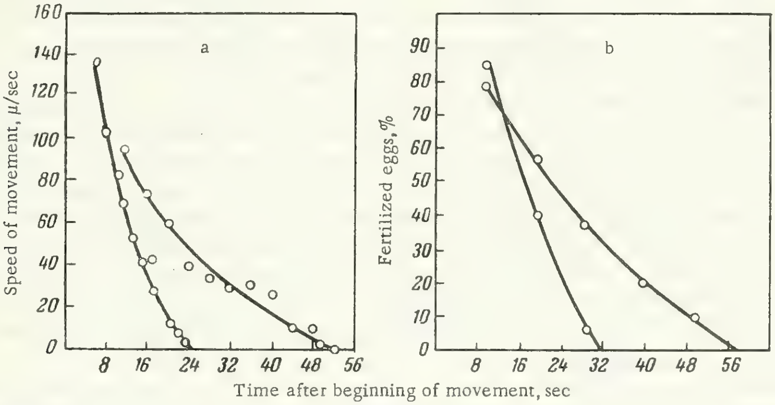

FIGURE 37. Speed of movement (a) and fertilizing capacity (b) of trout spermatozoa at various times after activation in water (after Schlenk and Kahmann, 1935).

Left-hand curves are for a temperature of 16°C; the right-hand curves for a lower (unspecified) temperature.

Schlenk and Kahmann believe that this is due to the retention of fertilizing capacity by isolated motile spermatozoa, whose movement was too slight to be recorded. However, it is possible that the period of movement of the spermatozoa under a slide in filming conditions may be several seconds shorter than in the insemination suspension.

As mentioned above, salmonid spermatozoa diluted in physiological solution and in cavity fluid have a considerably extended period of movement. Accordingly, the period in which a suspension of spermatozoa retains its fertilizing capacity is also extended. This was demonstrated for Salmo fontinalis (Werner, 1934) and lake trout Salmo trutta m. lacustris (Ginzburg, 1963a, b).

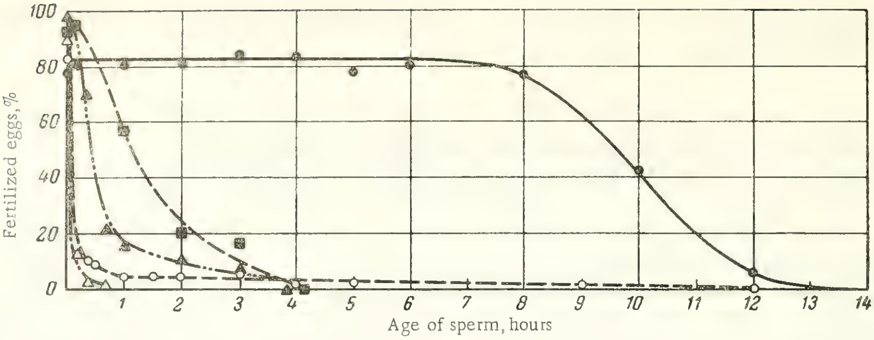

FIGURE 38. Retention of fertilizing capacity of spermatozoa of Acipenser güldenstädti colchicus in water.

Experiments with sperm from 5 males. Ratio of sperm to water, 1:9; temperature, 14.8–17.4°C.

Motility of the spermatozoon is not sufficient for fertilization, it must be capable of forward motion and capable of penetrating the micropyle on approaching it; wavering movement in one place does not ensure fertilization. This is clear from data on the duration of forward motion of the spermatozoa in lake trout, rainbow trout and pike (see Table 24).

It should be remembered that motility of the spermatozoa is not always a complete guarantee of fertilizing capacity. This was clearly demonstrated in mammals (guinea pig, mouse, etc.) in which spermatozoa lose their fertilizing capacity in the female genital ducts much earlier than their motility (see Bishop, 1961, p. 738). Motility may be retained and fertilizing capacity completely lost with certain pathological changes in spermatozoa, for example, in the case of the "headless" bull spermatozoa whose head is separated from the middle piece and tail which move intensively (Mann, 1961).

It should be noted that sperm of different males of a single species varies greatly in its properties.

The percentage of spermatozoa becoming motile when the sperm is diluted with water may vary over a wide range; all or almost all the spermatozoa are activated in some males, in others a larger or small portion and sometimes none. Cases of complete absence of spermatozoan activity in fresh ejaculate were noted for Alosa volgensis (Stroganov, 1936), brook trout (Allison, 1961), lake trout and sturgeons — Acipenser güldenstädti colchicus and A. stellatus (Ginzburg, unpublished data).

The duration of motility and fertilizing capacity in activated spermatozoa is also very variable. For example, in Acipenser güldenstädti colchicus it varied from 15–20 minutes to several or even many hours (Figure 38).

A graphic illustration of the significance of individual features of the sperm of different males is provided by the following experiment (Ginsburg, unpublished data). Aliquots of spawn from one stellate sturgeon female were inseminated with the thick, fluid or very fluid sperm of three males, containing, respectively, 3.25×10^9, 0.97×10^9, and 0.17×10^9 spermatozoa per cc sperm (in all three cases the sperm was well activated with water). Naturally, similar dilutions resulted in great differences in the percent of fertilization (Figure 39, a). The thick sperm gave maximum fertilization (about 95%) with a dilution of 1:1,000, whereas the same effect was achieved with the very fluid sperm only at a dilution of 1:3. Were differences in fertilization percentage due only to differences in the density of the suspensions, the curves would coincide in a graph drawn according to spermatozoan concentration. However, in such a graph (Figure 39, b) the distinct differences remained although the curves were close. In the case of the thick sperm, maximum fertilization was already obtained in a suspension containing 10^6 spermatozoa per cc. At the same concentration, the very fluid sperm gave only 33% fertilization, while 10^8 spermatozoa per cc were required for maximum fertilization. Insemination with fluid sperm of the second male gave intermediate results. Evidently, the difference in fertilizing capacity of the spermatozoan suspensions of the three males at similar concentrations is determined by differences in the properties of the spermatozoa themselves.

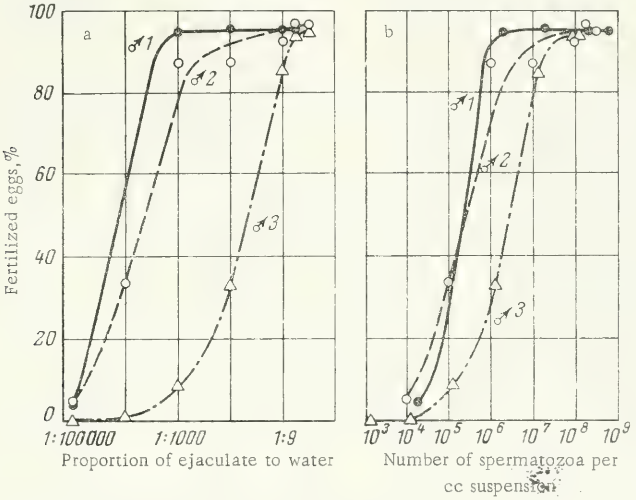

FIGURE 39. Individual variations in the fertilizing capacity of sperm in Acipenser stellatus:

a — percentage of fertilized eggs with similar dilutions of sperm from three males; b — ditto, calculated according to spermatozoan concentration in the suspension (temperature, 15.5—16.0°C).

2.4.4. Conditions for Prolonging Spermatozoan Fertilizing Capacity and the Possibility of Its Restoration after Temporary Loss

Spermatozoa in movement expend energy supplied by adenosine triphosphate (ATP), exactly as in contracting muscles. This was first demonstrated in mammalian spermatozoa in which inhibition of respiration and glycolysis rapidly exhausted ATP and movement of the spermatozoa ceased; these processes commence again, ATP is regenerated with the

TABLE 26. Retention of fertilizing capacity (in days) of "dry" sperm kept at various temperatures

Family	Species	Temperature, °C	Fertilizing capacity, determined according to:			Author
			motility of spermatozoa on addition of water	results of insemination		
				before great reduction in fertilization percent	at any fertilization percent	
Acipenseridae	Acipenser güldenstädti	1–4			5–8	Shmidtov, 1936
	Acipenser güldenstädti colchicus	On ice		Up to 5	Up to 8	Ginzburg, unpublished
		14.7–17.4			½–1½	
	Acipenser ruthenus	On ice	2			Arnol'd, 1915
		1–4	2–21		12	Shmidtov, 1936
	Acipenser stellatus	1–3		Up to 6	Up to 10½	Persov, 1941
Clupeidae	Clupea harengus harengus	4		2	>8	Blaxter, 1955
		7		>1	6	
Salmonidae	Oncorhynchus keta	2.5–5.8		1½	Up to 8	Barret, 1951
	Oncorhynchus gorbuscha	8–9		1½	2½	Withler and Humphreys, 1967
	Oncorhynchus nerka	2–8		1	3	Foerster, 1965
		8–9		½	4	Withler and Humphreys, 1967
	Salmo salar	5–6		2½	3	Brofeldt, 1923
	Salmo trutta m. lacustris	4–8	1–2			Scheuring, 1928
		0–1		2–15	4–15	Ginzburg, unpublished
		2.5–7.0		4.5	5–7	
		10.5–13.6		2	2	
	Salmo trutta m. fario	10–15			4–6	Henneguy, 1877, 1888
		4–8	1–5		3	Scheuring, 1928
		2.2–3.3			5	Butcher, 1944
		5	4–7			Dorier, 1951c
	Salmo hucho	8	2			Scheuring, 1928
	Salmo irideus				Up to 10	Scheuring, 1928
		5	4–11			Dorier, 1951b, c

TABLE 26 (Continued)

Family	Species	Temperature, °C	Fertilizing capacity, determined according to: motility of spermatozoa on addition of water	results of insemination: before great reduction in fertilization percent	results of insemination: at any fertilization percent	Author
	Salvelinus fontinalis	4–8	1–2			Scheuring, 1928
		5	4–10			Dorier, 1951c
	Coregonus lavaretus	6–8	5			Scheuring, 1928
		11–13	8			Medem et al., 1949
Percidae	Perca fluviatilis		6			Vrasskii, 1859
	Acerina cernua		6			Vrasskii, 1859

simultaneous recovery of motility of the spermatozoa (Engel'gardt and Burnasheva — see Engel'gardt, 1945, 1946; Lardy et al., 1945). Later experiments with sperm of Acipenser güldenstädti and A. stellatus established a connection between the ATP level in spermatozoa and their motility (Burnasheva, 1960). The spermatozoa were activated by the addition of water, and at the same time respiration increased sharply (in the first 2—3 minutes after activation the oxygen consumption was 37 mm^3/ml sperm/hour as against 17 mm^3/ml sperm/hour for unactivated spermatozoa). The ATP content also increased greatly from 30 to 104 μg/ml sperm. At this time, the spermatozoa moved actively. After 5—10 minutes, ATP content decreased to almost the original value and the intensity of movement declined sharply.

Immotile spermatozoa, characterized by a low metabolic level, retain for long their energy reserves and, correspondingly, the capacity for becoming activated and for fertilizing when external conditions change.

The fertilizing capacity of stored "dry" sperm — ejaculate without added water in which spermatozoa are immotile — is retained for many hours or even days (Table 26). The length of retention of this capacity depends largely on the storage temperature. Dry sturgeon sperm of good quality kept at temperatures from 0—35°C showed a decrease in the fertilizing capacity within a short time (60—100 minutes) only at the highest temperatures (Ginzburg, unpublished data, see p. 86 and Figure 21, a', b'). However, the temperature effect is very clear in sperm kept for longer periods. Thus, sperm of several males of Acipenser güldenstädti colchicus retained the fertilizing capacity for no more than 12—36 hours at

14.7–17.4°C, and for up to 8 days when kept on ice. Similarly, sperm of several lake trout retained fertilizing capacity for 2 days at 10.5–13.6°C and from 4 to 15 days at close to 0° (in a refrigerator).

The variation in sperm quality in different males is also reflected in the duration of retention of fertilizing capacity at lowered temperatures. Thus, lake trout sperm in which only a small percentage of spermatozoa were activated usually loses it fertilizing capacity within 1–2 hours when placed in ice. Good-quality sperm can retain this capacity for several, sometimes many days; in one case 96% fertilization was obtained after sperm had been kept for 15 days at 0°C (Figure 40).

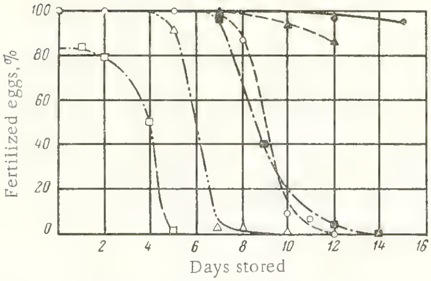

FIGURE 40. Retention of fertilizing capacity by "dry" sperm of 6 lake trout kept in a refrigerator (close to 0°C)

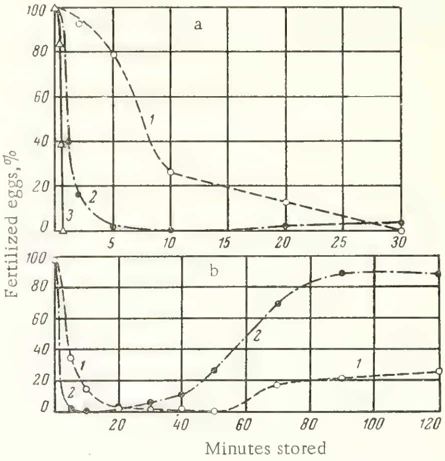

FIGURE 41. Change in fertilizing capacity of lake trout sperm stored in mixtures with cavity fluid (1), Ringer's solution (2) and water (3).
[Kinetics of loss of fertilizing capacity (a); reactivation of fertilizing capacity on addition of water (b).]

Deep-freezing considerably prolongs retention of the fertilizing capacity of sperm, and is used practically with poultry and livestock.

An initial experiment of this method with fishes (Sneed and Clemens, 1956) was unsuccessful; only 20% of the spermatozoa were activated in carp sperm frozen for 60 hours at -73°C in isotonic Ringer's solution containing 6–12% glycerin.

Considerably better results were achieved (Blaxter, 1955) on freezing the testes of Atlantic herring. Small pieces of testes were placed in test tubes containing 12.5% glycerin in diluted seawater (1 part distilled water to 4 parts seawater) and frozen rapidly, then stored on dry ice (-79°C) under a vacuum. Spermatozoa in the testical tissue kept under such conditions retained their fertilizing capacity for 6 months without an appreciable decrease; the frozen spermatozoa fertilized 85% of the eggs. Spermatozoa frozen for 6 months were used for crossbreeding spring-spawning and fall-spawning races of Atlantic herring.

Spermatozoa, which were first activated but then quickly ceased moving and remained inactive, also may apparently retain their fertilizing capacity for a long period. This is indicated by experiments with sperm preserved with ethanol.

According to the data of Lebedintseva and Nedoshivina (1912), trout sperm suspended in 1% ethanol retains its fertilizing capacity for 7 days. However, Scheuring (1925, p. 216), repeating the experiment on trout with 1% ethanol, obtained a rapid loss of the sperm's fertilizing capacity.

Sterlet sperm (Shmidtov, 1936) in a 2% ethanol solution showed active movement of the spermatozoa, which soon ceased. Spermatozoa in this suspension retained fertilizing capacity for 17–20 days.

Spermatozoa, having swum for a long time, cease moving and remain inactive, but can also be reactivated. This was demonstrated in sperm of Echinometra subangularis (Southwick, 1939) in which movement of spermatozoa was restimulated by the addition of seawater. Movement of the reactivated spermatozoa lasts for a short time, but can again be activated repeatedly with progressively shorter swimming periods.

Spermatozoa of lake trout also can be reactivated after a long swimming period (Ginzburg, 1963a, b, unpublished data). Sperm aliquots from a single male were mixed with cavity fluid, Ringer's solution and water and incubated with periodic testing of the fertilizing capacity of the suspension. Uniform results were obtained in 6 experiments, two of which are shown in Figure 41. A gradual decrease in fertilizing capacity, followed by its complete loss, appears in cavity fluid after 20–30 minutes, in Ringer's solution after 5–10 minutes and in water in the first minute (Figure 41, a).

On just losing the fertilizing capacity, spermatozoa are not reactivated by the addition of water. Only after a given "rest period" (40–60 minutes after mixing with cavity fluid, 20–40 minutes after mixing with Ringer's solution) is the fertilizing capacity of the sperm partially restored (Figure 41, b). On reactivation, sperm diluted with Ringer's solution consistently gave a high fertilization percentage (90% in the experiment shown in Figure 41, b); sperm kept in cavity fluid always gave less complete restoration of fertilizing capacity. The capacity for reactivation is retained for more than a day. Salmo salar sperm in cavity fluid was activated after 2 or 3 days (Ellis and Jones, 1939). Evidently, energy reserves are gradually renewed after cessation of motion and make reactivation possible when water is added.

Trout spermatozoa kept in water did not recover fertilizing capacity, apparently because of the irreversible structural changes mentioned above. The functional structure of the spermatozoon is conserved for a long period in cavity fluid and physiological solution which is the basis for restoration of the capacity for activation.

Chapter 3

GAMETIC ASSOCIATION

Gametic association is the initial and very vital link in the process of fertilization. Until recently hardly any information was available concerning the mechanism of the association of the gametes. The gap in factual knowledge had to be compensated for by hypotheses based on indirect evidence.

The first hypothesis concerning sperm-egg association was advanced by F.R.Lillie (1913a, b, 1914, 1919). Lillie established that eggs of the sea urchin Arbacia and of the polychaete Nereis emit into the water a substance (or substances) which activate, aggregate and agglutinate the spermatozoa. Lillie called the agglutinating substance produced by the eggs "fertilizin," i.e., the fertilizing substance, stressing the central role which, in Lillie's opinion, fertilizin plays in this process. Lillie theorized that the gametic association is a reaction between three components — the spermatozoon, the fertilizin and the egg. To illustrate the mechanism of sperm-egg association Lillie used an immunological concept (theory of lateral chains and amboceptor of Paul Ehrlich).

According to Lillie, the fertilizin found in egg water (water in which unfertilized eggs have been kept for some time) originates in the cortical layer of the egg. He suggested that fertilizin has two lateral chains — spermophilic and ovophilic (Figure 42).

According to this theory, the spermatozoan head surface bears receptors which attach to the spermophilic chain of fertilizin and activate it. The ovophilic lateral chain of the activated fertilizin associates with the receptors of the egg, thus realizing the sperm-egg association. In the absence of fertilizin, fertilization is impossible. Besides fertilizin and receptors linking ovophilic groups, the cortical layer of the egg contains a substance capable of blocking the spermophilic groups of fertilizin, called "antifertilizin" by Lillie. After association of the spermatozoon with the egg, the free molecules of fertilizin are blocked by the antifertilizin. Furthermore, the cortical layer loses its receptivity to spermatozoa, and thus the egg is protected from polyspermy. Fertilizin can interact with receptors of the spermatozoa not only in sperm-egg association but also outside the egg, causing agglutination.

The orderly theory of Lillie had many merits. With a single mechanism, it explained the agglutination of the spermatozoa, sperm-egg association and the block to polyspermy; the specificity of fertilizin substantiated species- and tissue-specificity of fertilization, as well as the nonspecific character of parthenogenetic agents which function like fertilizin. Not surprisingly, this theory attracted considerable attention and served as a starting point for numerous investigations.

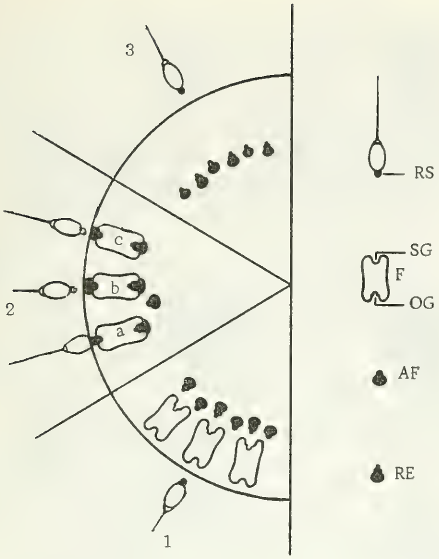

FIGURE 42. Diagram illustrating Lillie's fertilizin theory of fertilization (Lillie, 1914):

1 — distribution of substances in unfertilized egg and spermatozoon; 2 — mechanism of normal fertilization: spermatozoon receptor binds to spermophilic group of fertilizin, leading to its activation and union of the ovophilic group of fertilizin (a) with the egg receptor; molecules of antifertilizin bind with spermophilic groups of neighboring fertilizin molecules (b, c), blocking supernumerary spermatozoa; while fertilizin molecules (b, c) unite with egg receptors; 3 — illustrating the impossibility of sperm-egg association when fertilizin is lost. AF — antifertilizin; OG — ovophilic group of fertilizin; RS — spermatozoon receptor; RE — egg receptor; SG — spermophilic group of fertilizin; F — fertilizin.

Substances produced by the eggs and causing agglutination of homologous spermatozoa — fertilizins according to Lillie's terminology — have been found in many but not all animals studied. Among vertebrates, they were found in Cyclostomi, fishes and mammals. Eggs of various animals excrete a substance, antifertilizin, which inactivates fertilizin. A similar substance was also discharged from spermatozoa. The latter substance, called fertilizin of the spermatozoon, was suggested to be the surface receptor of the spermatozoon, which binds with the fertilizin of the egg on fertilization.

Furthermore, a large amount of data have contradicted Lillie's theory. Exceptions to this theory and its defenses and additional hypotheses have been dealt with in special reviews (Tyler, 1948, 1959; Metz, 1957a; 1961; Dorfman, 1963), and will not be discussed here. However, the basic position of this theory that fertilizin is essential for fertilization has not been confirmed. This will be dealt with in more detail in the section on localization, chemical nature and function of fertilizin (p. 163).

After many years of study of the specific substances produced by the gametes, as well as direct investigations into the process of gametic association, Lillie was compelled to revise the fertilizin theory of fertilization. In recent years, it has been replaced by a theory of specific pinocytosis or phagocytosis elaborated by Tyler (1959, 1961, 1962a, b, 1963, 1964). Like Lillie, Tyler conceived the sperm-egg association as a result of interaction of specific receptors localized at the surface of the egg and sperm head (fertilizin of the egg and antifertilizin of the spermatozoon). This interaction is based on a complementary chemical configuration of receptors of the "lock and key" type (similar to antigen and antibody reactions). Tyler suggested that the sperm head comes in contact with the microvilli which penetrate the vitelline membrane and carry reactive groups of fertilizin (Figure 43). The vitelline membrane is lyzed in the region of contact with the sperm head. The area of contact between the surface membranes of the microvilli and sperm head increases rapidly (they are linked by means of reactive receptor groups — numerous "keys and locks" — similarly to interlocking of a zipper). The microvilli surround the sperm head, drawing it into the cytoplasm at the same time. The folds of the surface membrane, which remains continuous, close above the spermatozoon as in pinocytosis and phagocytosis. After the fertilizing spermatozoon has established contact with the egg cytoplasm, the rest of the microvilli in the egg surface are resorbed, drawing the reactive groups of fertilizin away from other spermatozoa and making their penetration impossible.

Taking into account recent data on variably-shaped microvilli in the egg surface and on the membrane impermeable to spermatozoa, Tyler suggested a new hypothesis concerning the gametic association and the block to polyspermy. This hypothesis has principles in common with the fertilizin theory of fertilization, but includes new concrete concepts which, particularly important, permit direct verification.

Tyler's theory is not only disproved by the fact that the presence of fertilizin is not an essential condition of fertilization, but is also disproved by direct morphological studies of the process of gametic association. Stepwise electron micrographs of the gametic association clearly showed that this process differs in principle from pinocytosis and phagocytosis. The spermatozoon penetrating the cytoplasm is never surrounded by a continuous surface membrane of the egg. The function attributed by Tyler to the microvilli in sperm-egg association has not been confirmed; in some forms, such as Saccoglossus kowalevskii, the microvilli do not generally protrude above the surface of the vitelline membrane before fertilization (Colwin and Colwin, 1963a, 1964).

It seems to me that the time for speculative hypotheses and theories of fertilization is past. Due to the possibilities offered by the electron microscope, much has been learned in the last decade concerning the fertilization process and an understanding of its mechanism has been approached. In the first two sections of this chapter, the results of direct morphological investigations of gametic association will be discussed. The first section is devoted to the initial phase of this process, including formation of the acrosomal filament (or tubule) and the contact it establishes between the spermatozoon and the cortical layer of the egg. The second section is devoted to the second phase of gametic association, i. e., the actual penetration of the spermatozoon into the cytoplasm. The third section deals with the specific substances contained in the gametes and affecting their association. Of all factors involved in gametic association, these substances have merited the most attention of investigators until recently. Finally, the last two chapters deal with more particular questions: the significance of the concentration of spermatozoa at insemination and the time required to effect the sperm-egg encounter. Although these questions are of great practical importance, they have until recently been elaborated principally for mammals (Austin and Bishop, 1957a, b; Milovanov, 1962) while there are only fragmentary data for fishes and other animals. Therefore, the fifth and sixth chapters contain original data obtained for sturgeons and salmonids and published only in part.

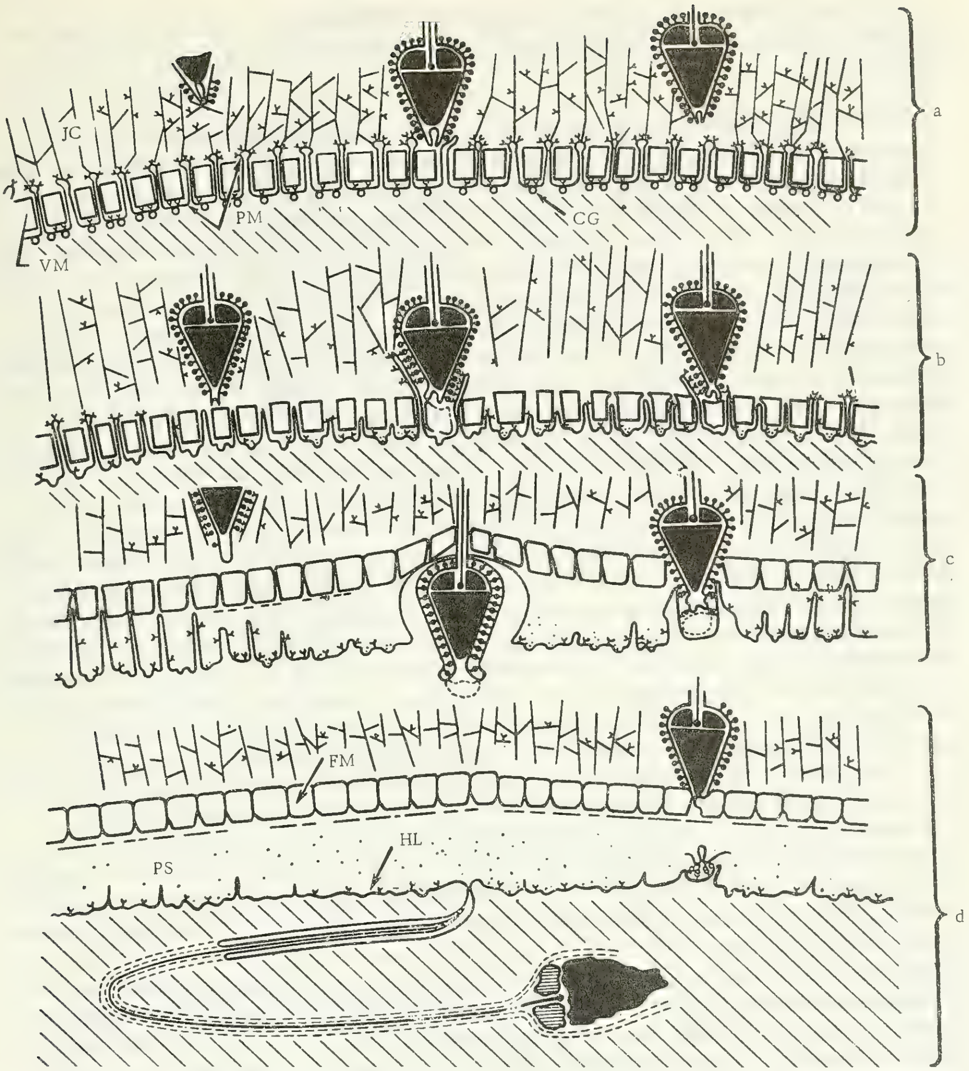

FIGURE 43. Diagram illustrating sperm-egg association based on interaction of fertilizin and antifertilizin and of specific pinocytosis (after Tyler, 1962 a, b):

a—d — Consecutive stages of sperm penetration. The reactive groups of antifertilizin are shown as club-shaped structures at the surface of the spermatozoon; reactive groups of fertilizin appear as cup-shaped structures on the plasma membrane (PM), which forms microvilli, and micelles of the jelly coat (JC) which bind because of complementary configuration, as in antigen-antibody reactions. After the spermatozoon establishes contact with the vitelline membrane (VM) (a), several microvilli envelop the sperm head (b); the acrosomal vacuole opens and releases a lysin which dissolves the section of the vitelline membrane between the microvilli enveloping the sperm head. The union of the microvilli and sperm head surfaces resembles interlocking of a zipper, and the spermatozoon is drawn into the egg (c). Some after this, the fused membranes of the spermatozoon and egg disintegrate and disappear (d). The rapid resorption of the remaining microvilli on the egg surface inhibits penetration of spermatozoa which later approach the egg (spermatozoon on the right, in c and d), and renders the plasma membrane receptor of the egg inaccessible to other spermatozoa (spermatozoon on the left in c). Legend: HL — hyaline layer; VM — vitelline membrane; CG — cortical granule; FM — fertilization membrane; PM — plasma membrane; PS — perivitelline space; JC — jelly coat.

3.1. FIRST PHASE OF GAMETIC ASSOCIATION — SPERMATOZOAN ACTIVATION AND ACROSOMAL REACTION

The contact between the gametes causes changes in the spermatozoon, affecting first and foremost the acrosome and called the acrosomal reaction. Study of these changes became possible only within the last few years with the aid of phase-contrast microscopy of living objects and electron microscopy. The acrosomal reaction was established and investigated for echinoderms (Dan, 1952, 1954a, 1960; Dan et al., 1962; 1964; Tyler, 1952; Rothschild and Tyler, 1955; Metz and Morril, 1955; Colwin and Colwin, 1955a, 1956; Afzelius and Murray, 1957; Bernstein, 1962; Franklin, 1965), mollusks (Dan and Wada, 1955; Rothschild and Tyler, 1955; Niijima and Dan, 1965a, b), polychaetes (Colwin and Colwin, 1955b, 1961b; Colwin et al., 1957; Metz and Morril, 1955), enteropneusts (Colwin and Colwin, 1963a; Colwin et al., 1957), Cyclostomi (Afzelius and Murray, 1957; Kille, 1960a), sturgeons (Detlaf and Ginzburg, 1963) and amphibians (Kille, see Dan, 1960).

Let us begin with a brief description of the basic features of the acrosomal reaction, which has been studied in greatest detail in marine invertebrates (see reviews: Dan, 1956; Colwin and Colwin, 1957a, 1964; Metz, 1957b; Allen, 1958; Dorfmann, 1962, 1963). The egg membranes of these invertebrates do not contain micropyles, so that the spermatozoon must pass through the membranes in order to reach the cytoplasm. The acrosomal reaction represents the mechanism of the spermatozoon for overcoming this barrier.

To illustrate the changes in the acrosomal region occurring upon contact with the egg we shall take as an example the sperm-egg association in annelid Hydroides hexagonus and in an enteropneust Saccoglossus kowalevskii, which were studied in detail in the brilliant investigations of the Colwins (Colwin and Colwin, 1960, 1961a, b; 1963a, 1964; Colwin et al., 1957). Details can be found elsewhere, and only the basic processes will be mentioned here. There is an acrosomal vacuole at the top of the sperm head of these animals (Figure 44, a, a') bounded by the acrosomal membrane and containing an acrosomal granule. In the region of contact with the nucleus, the acrosomal membrane forms outpouchings (one in Saccoglossus, several in Hydroides). The acrosomal vacuole is covered by the spermatozoan plasmalemma. In the first few seconds after contact with the egg membrane, an opening appears at the top of the sperm head (Figure 44, b, b') along whose margin the acrosomal membrane joins the plasmalemma to form a single uninterrupted surface. The acrosomal granule is thus exposed and its material rapidly disperses while the adjoining section of the egg membrane is lysed (the juxtaposition of these processes indicates the localization of spermatozoan lysin in the acrosomal granule — see p. 169). The outpouchings of the acrosomal membrane grow in size, and form acrosomal filaments (one in Saccoglossus or a whole cluster in Hydroides). As these filaments lengthen, the acrosomal membrane is extruded so that its initially internal surface faces the acrosomal filaments (Figure 44, c, c'). The single filament of Saccoglossus and the several filaments of Hydroides penetrate through that lysed section of the egg membrane to the plasma membrane of the egg. This contact is established 7—9 seconds after insemination, sometimes even earlier. At the point of contact the plasma membranes of the spermatozoon and egg fuse, and the contents of the acrosomal filament unite with the ooplasm (Figure 44, d, d') forming a single cell, the zygote of the two gametes .

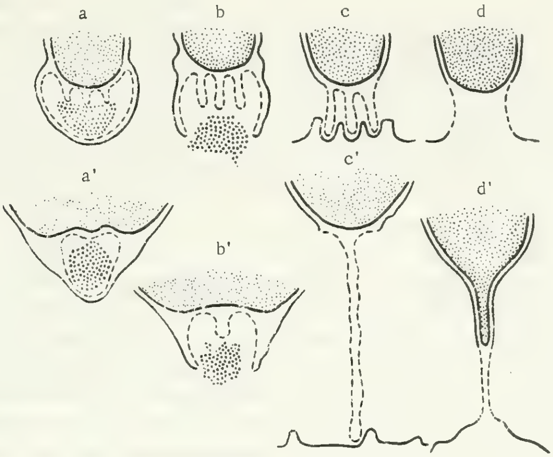

FIGURE 44. Diagram of sperm-egg association in Hydroides hexagonus (a—d) and Saccoglossus kowalewskii (a'—d') (after Colwin and Colwin, 1964).

Egg membranes are not shown. The dotted line indicates the acrosomal membrane, large dots indicate the acrosomal granule; a, a' — acrosomal region of spermatozoon before encounter with egg; b, b' — formation of opening at top of sperm head, dispersion of acrosome granule, lengthening of acrosome filaments; c, c' — plasma membrane of spermatozoon (wall of acrosomal filaments) contacts plasma membrane of egg; d, d' — union of plasma membranes of spermatozoon and egg.

The structure and changes of the acrosomal apparatus in the sea urchin (Afzelius, 1955; Dan et al., 1962, 1964; Franklin, 1965) and the bivalve mollusk Mytilus edulis (Niijima and Dan, 1965a, b) are similar in the main to those described above. During the acrosomal reaction an opening appears at the top of the sperm head exposing the contents of the acrosomal vacuole. At the margin of the opening the acrosomal membrane and plasmalemma merge and the contents of the acrosomal vacuole are dispersed. The acrosomal outpouching (or filament, according to the Colwins' terminology) emerging from the acrosomal wall in the region of its contact with the nucleus and covered by the inverted acrosomal membrane, lengthens so that its tip reaches the egg surface. Thus, it appears that in all cases contact with the egg membrane is made by a newly formed part of the sperm head surface (originally, the inner surface of the acrosomal membrane).

The basic similarity of the main processes in the first phase of gametic association in representatives of four systematically distant groups of animals suggests that the transformations in the acrosomal system of the spermatozoon have a more general significance (see Colwin and Colwin, 1964).

The length of the acrosomal filaments varies greatly even within a single phylum. In most sea urchin species studied the acrosomal filament was only from 0.5μ to several microns in length, but it reached 15—28μ in starfishes and up to 75—90μ in the holothurian Thyone briareus. These variations are associated with the thickness of the egg membrane barrier. In sea urchins the outer jelly coat of the egg consists of friable mucus, so that the spermatozoon easily swims in it and the acrosome only pierces a thin vitelline membrane. In starfishes and holothurians, the external egg membrane consists of a dense gelatinous substance, which the spermatozoon cannot possibly penetrate by swimming movements. Accordingly, acrosomal filaments are longer in these animals than in sea urchins, and the length of the filaments corresponds approximately to the total thickness of the membranes.

In echinoderms, changes are observed in the middle piece and tail along with changes in the acrosome (Figure 45). The previously flattened middle piece becomes nearly spherical and is displaced to one side relative to the point of emergence of the flagellum. Furthermore, the attachment to the flagellum is weakened and sometimes it separates completely (not the whole middle piece divides off, but only the mitochondrial sheath while centrioles remain in place). Separation of this part during the acrosomal reaction may be connected with the fact that during normal fertilization it does not penetrate the egg (Tyler, 1952). There is also a change in the position of the flagellum with respect to the sperm head; originally it is oriented along the anterior-posterior axis of the head, but during the acrosomal reaction it almost forms a right angle to it (Figure 45, b).

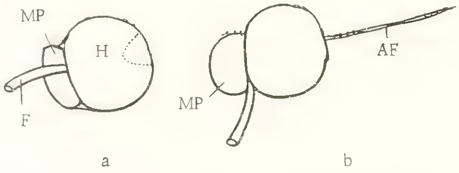

FIGURE 45. Changes in starfish spermatozoon due to acrosomal reaction:

a — initial condition; b — reacting spermatozoon (after Dan, 1956). AF — acrosomal filament; H — head; MP — middle piece; F — flagellum.

The changes in the spermatozoon on encountering the egg are very considerable and mark the beginning of a whole chain of transformation which end with complete fusion of the gametes. Analogous changes of the egg involve the discharge of the cortical granules and formation of the perivitelline space. Therefore, I believe that Colwin and Colwin (1963a) are correct in regarding the acrosomal reaction as a manifestation of activation of the spermatozoon, similar to activation of the egg.

All the described changes in the spermatozoon can occur not only on contact with unfertilized eggs, but generally also when influenced by egg water and certain nonspecific agents — alkaline seawater (pH about 9), excess calcium, contact with a hard surface (for example, a glass slide).

Sometimes spermatozoa eject the acrosomal filament in ordinary seawater, but this is rare. The mature spermatozoon represents a highly sensitive system prepared to undergo the acrosomal reaction; therefore, it responds to nonspecific stimuli, much as the mature egg is activated by such stimuli and as a muscle fiber contracts.

The acrosomal reaction requires calcium in the medium. In calcium-free seawater no ejection of acrosomal filaments occurs in sea urchins (Dan, 1954b), and only the mitochondrial sheath of the middle piece rounds off and shifts (changes which usually accompany formation of the acrosomal filament). Exceptionally, the acrosomal reaction may occur in the sea urchin Clypeaster japonica in a calcium-free medium if magnesium ions are present (Dan, 1956).

Among vertebrates, a typical acrosomal reaction was found in Cyclostomi and sturgeons.

In the lampreys Lampetra fluviatilis and L. planeri (Kille, 1960a), as in the marine invertebrates mentioned above, egg membranes lack micropyles. The spermatozoa penetrate the egg in the region of the animal pole where the thick two-layered chorion is covered by a gelatinous substance pierced by thin radial fibrils (so-called "Flocke"). The spermatozoa swim through the gelatinous mass and penetrate the outer layer of the chorion. The acrosomal reaction serves to overcome the inner layer of the chorion. The spermatozoon ejects a flexible acrosome filament with a length of about 50μ and with a thickness of 0.0025μ (Kille, 1960a) – 0.006μ (Afzelius and Murray, 1957).

The lamprey spermatozoon not only ejects its acrosomal filament on contact with an egg and when affected by egg water, but also regularly in ordinary freshwater (in suspension), on contact with a hard surface and in fixing solution. The unusual ease with which the acrosomal reaction is stimulated in the lamprey is apparently due to acrosomal structure; the mature spermatozoon apparently contains a fully formed acrosomal filament which appears in electron micrographs to be helical and pierces the nucleus throughout its length from top to base (Kille, personal communication; Follenius, 1965).

Considering the great size of the acrosomal filament and the fact that it is easily ejected under most diverse conditions, the acrosomal filament of the lamprey spermatozoon was long ago described as the "head bristle" (Kopfborste) of the spermatozoon (Ballowitz, 1904, 1913; Retzius, 1921). At that time this formation appeared unique.

In selachians, as in the Cyclostomi, egg membranes are permeable to sperm. The selachian spermatozoa have a well developed acrosome which can be suggested to play the usual role in penetration of the egg. Since insemination in these fishes is internal and the gametic encounter occurs in the genital tract of the female, it has been very difficult to study the changes taking place in the spermatozoon and nothing is known of them at present.

The dense egg membranes of sturgeons are impermeable to spermatozoa, which reach the cytoplasm through micropylar canals. The large size of the eggs and the opaqueness of the membranes make it impossible to observe the gametic association with a light microscope. To study changes in the spermatozoa of Acipenser güldenstädti and A. stellatus during gametic association, spermatozoa were exposed to egg water and then examined in a phase microscope and ultramicroscopically (Detlaf and Ginzburg, 1963). Under these conditions a large proportion of the spermatozoa (60—90% in separate experiments) ejected acrosomal filaments. In a small percentage of cases, activated spermatozoa may also be found on dilution of the ejaculate with ordinary river water. The acrosomal filament (Figure 46) is generally as long as the rod-shaped sperm head (4—6μ in A. stellatus, 5—8μ in A. güldenstädti), but sometimes it is considerably longer. The longest acrosomal filament in A. güldenstädti was 14μ long. The thickness of the filament in different spermatozoa varies between 0.02 and 0.08μ. Sometimes the filament divides at the end (Figure 47, a) or close to the base (Figure 46, c); in some spermatozoa there were 2—3 acrosomal filaments emerging independently from the top of the sperm head (Figure 47, b, c). Sometimes the acrosomal filament splits along a certain length into 2—3 branches, which reunite in a single filament. The base of the acrosomal filament is often thickened, reaching 0.2—0.4μ in width. The widened base of the filament is clearly visible in the phase microscope, but most of the thin part of the filament is indiscernible There is sometimes an expansion (Figure 46, a) varying in size and shape at the tip of the filament.

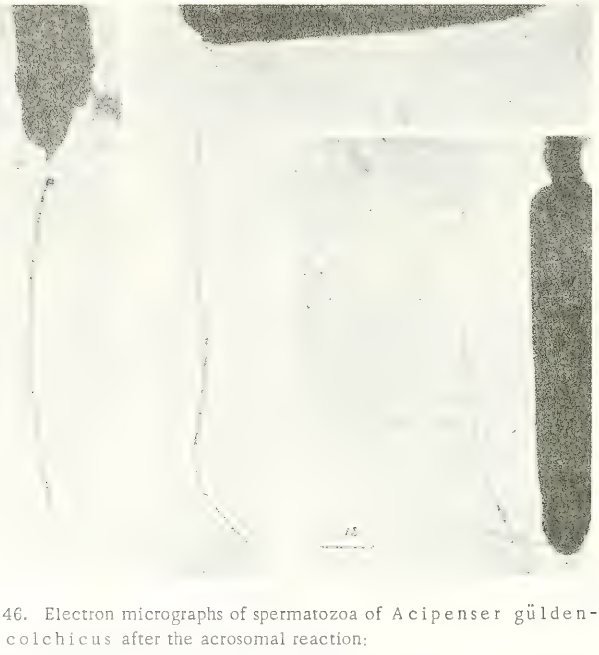

FIGURE 46. Electron micrographs of spermatozoa of Acipenser güldenstädti colchicus after the acrosomal reaction:

a — acrosomal filament with expansion at tip (broken at base); b — long, thin acrosomal filament; c — branching acrosomal filament (after Detlaf and Ginzburg, 1963).

Ejection of the acrosomal filament in sturgeons, as in the sea urchin and Saccoglossus, occurs very rapidly. Several sturgeon spermatozoa fixed 10 and 30 seconds after being placed in egg water, already had acrosomal filaments of typical form and size.

Regular changes are also observed in middle pieces of spermatozoa with an acrosomal filament. The mitochondrial sheath rounds and swells to reveal a granular structure, and shifts laterally relative to the point of emergence of the tail. Meanwhile, the union with the flagellum becomes weaker, and a clump of middle-piece material is transferred to the lateral surface of the head or torn away completely. The transformations described are very similar to changes in echinoderms' spermatozoa, except that the orientation of the flagellum relative to the sperm head does not change in sturgeons.

Swelling and shifting of the middle piece is observed not only in spermatozoa with an acrosomal filament, but also in those with no visible changes in the head.

The same work indicated that formation of an acrosomal filament in Acipenser güldenstädti and A. stellatus requires calcium ions, which may be replaced by other bivalent cations of the same group — magnesium or strontium (but not barium or manganese) ions. It is interesting that only strontium ions fully replace calcium ions in the sperm-egg association; these ions not only ensure the formation of the acrosomal filament, but also fertilization. On the other hand, fertilization does not occur if only magnesium ions are present, although the spermatozoon is capable of ejecting a typical acrosomal filament. Apparently true gametic association fails in this case because the acrosome does not adhere to the egg surface.

A special feature of the acrosomal reaction in sturgeons are the diverse shapes of the acrosomal filaments — the great variations in filament length, frequently observed branching and the formation of several acrosomal filaments diverging in different directions from the head. This polymorphism of the filaments is apparently connected with the conditions of the gametic association. Since the acrosomal filament serves as means of penetrating the egg membrane, evolutionary selection has eliminated spermatozoa with branching and numerous filaments and preserved spermatozoa forming a single unbranched filament whose length is equal to the egg membrane thickness. In sturgeons the acrosomal reaction has lost its original significance since the spermatozoon penetrates to the cortical layer of the egg via the micropylar canal. Therefore, variations in shape of the acrosomal filament do not impede gametic association, and spermatozoa with branching and numerous filaments are not eliminated in selection.

Among the many primitive features preserved in sturgeons, there is also the primordial mechanism of gametic association. Despite development of dense membranes and micropyles, gametic association in sturgeons involves the acrosomal reaction. Spermatozoa of the more specialized teleosts, which also have egg membranes with a micropylar system, have lost the acrosome during evolution.

This mechanism of gametic association is inherent among vertebrates, as far as we know at present, only in certain fishes. The egg membranes of amphibians and all amniotes are permeable to sperm and do not have micropylar canals. Accordingly, these groups retain an acrosome in the spermatozoon, and it may be assumed that it participates in overcoming the barrier separating the spermatozoon from the egg cytoplasm. Unfortunately, sperm-egg association in amniotes has not yet been studied sufficiently to provide more than scant data on changes in the acrosomal region.

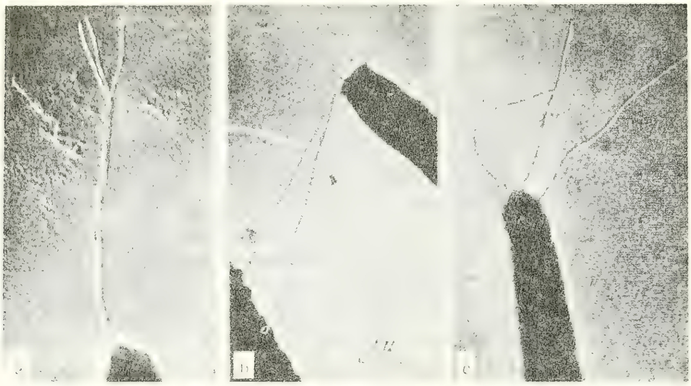

FIGURE 47. Electron micrographs of spermatozoa of *Acipenser stellatus* with one branching (a) and several (b, c) acrosomal filaments.

Shadowed by chromium.

In the frog *Discoglossus pictus*, the spermatozoon apparently forms a long acrosomal filament (Kille, see Dan, 1960). There are no grounds for regarding the granule arising at the end of the sperm head before the spermatozoon separates from the Sertoli cell as analogous to the acrosomal filament of sea urchins, as did Favard (1955). The same work described changes in spermatozoa of *Rana temporaria* when placed in freshwater which are possibly connected with the acrosomal reaction.

In recent years structures resembling the acrosome of invertebrates have also been described for mammals. Spermatozoa of the bull and rabbit have a conical apical body at the tip of the sperm head, which represents a special part of the acrosome (Blom and Birch-Andersen, 1961), or a separate formation covered with acrosomal material (Hadek, 1963c) containing round granule or vacuole. This formation is easily destroyed and is discernible only in live spermatozoa.

Light microscope studies of penetration by rabbit, swine and sheep spermatozoa through the zona pellucida revealed in many cases a thin filiform structure arising from the top of the sperm head and resembling the acrosomal filament of several invertebrates (Dickmann, 1964; Dickmann, and Dziuk, 1964; Dziuk and Dickmann, 1965). Ultramicroscopic study of rabbit spermatozoa revelaed no such structures (Hadek, 1963a, c). On approaching the egg surface closely, sperm heads lack a cell membrane, acrosome or head sheath, whereas the exposed apical body is markedly elongated in the shape of a narrow cone and not a filament. At this time the apical body resembles in form the acrosome of the sea urchin spermatozoon.

3.2. SECOND PHASE OF GAMETIC ASSOCIATION – PENETRATION OF SPERMATOZOON INTO OOPLASM

A description of spermatozoan penetration into the egg cytoplasm in marine invertebrates will be given first since these groups have been studied most thoroughly. From the moment the acrosomal filament (or tubule) reaches the cortical layer and becomes associated with it, the egg is stimulated to develop and the second phase of the process of gametic association begins. During this phase, the spermatozoon passes through the membranes and is absorbed by the cytoplasm. In the first phase of gametic association the spermatozoon was active, whereas in the second phase it is usually immobile and the egg becomes activated. The activated cytoplasm flows around the acrosomal filament and, in many animals, forms the fertilization cone already known to early investigators (see Rothschild, 1956; Colwin and Colwin, 1957a). Via the acrosomal filament, the spermatozoon is drawn passively into the egg through the membranes and is absorbed by the cytoplasm. The mechanism drawing in the spermatozoon is not clear. However, it is certain that the acrosomal filament does not contract (the length and diameter of the filament remain unchanged) and the fertilization cone does not retract. In some cases (e.g., in Holoturia atra), the fertilization cone begins to decrease in size only after the spermatozoon has penetrated the egg (Colwin and Colwin, 1955a, 1957a).

The second phase of gametic association lasts much longer than the first. In Saccoglossus kowalevskii, the first phase lasts seconds, while the second phase takes several minutes (Colwin et al., 1957).

An important roll in the second phase is played by surface membranes of the gametes. Investigations of Hydroides and Saccoglossus (Colwin and Colwin, 1961c, 1963b, 1964) showed that the region of contact between the acrosomal filaments and the cortical layer, the plasma membranes of the gametes, fuse into the single surface membrane of the zygote. The latter membrane thus has a mosaic structure; most of it is derived from the egg, while a small section is derived from the spermatozoon. Only the nucleus and other internal organelles enter the egg cytoplasm (Figure 48). Similar phenomena are observed in gametic associations in Prasiola (Friedman, 1962) and the rat (Szollosi and Ris, 1961).

In the lamprey, like the invertebrates studied, implantation of the tip of the acrosomal filament in the cytoplasm activates the egg and induces the cortical reaction (Kille, 1960a). At this moment, the active movements of the spermatozoon cease. The perivitelline space begins to be formed 15 seconds (at 18°C) after the spermatozoon reaches the chorion. When the egg surface in the region of the animal pole emerges from the membranes, the acrosomal filament appears, enveloped by a thin sheath of cortical cytoplasm. Within $2\frac{1}{2}$ minutes the sperm head begins to advance slowly through the inner layer of the chorion, then crosses the perivitelline space much more rapidly and within 3 minutes is absorbed in the ooplasm.

If the ooplasm of the lamprey egg is separated from the dense membranes and the latter inseminated, spermatozoa enter the jelly coat, reach and stick to the outer layer of the chorion, but never penetrate through the inner layer. This proves that the cytoplasm participates actively in drawing the spermatozoon into the egg.

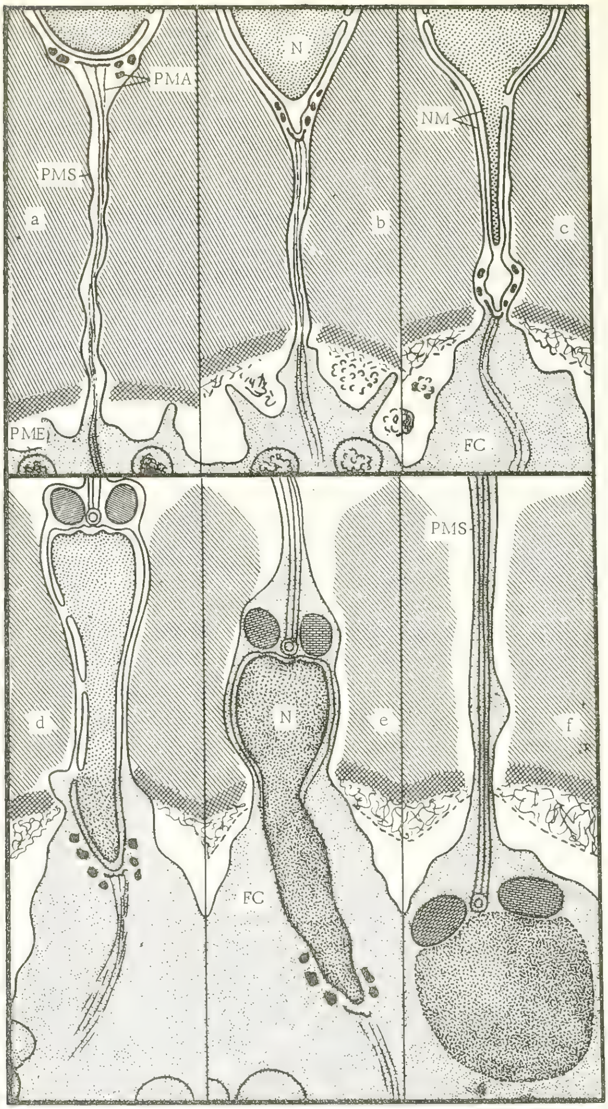

FIGURE 48. Diagrams of gametic association of Saccoglossus kowalevskii:

a — acrosomal filament fuses with egg cytoplasm, after fusion of spermatozoan and egg plasma membranes; b, c — nucleus of spermatozoon is drawn out in the direction of the fertilization cone; d, e — cytoplasm penetrates between internal structures of spermatozoon and its plasmalemma; f — head and middle piece of spermatozoon engulfed in egg cytoplasm, fertilization cone retracts (after Colwin and Colwin, 1963b). FC — fertilization cone; PAM — periacrosomal material; PMS — plasmalemma of spermatozoon; PME — plasma membrane of egg; N — nucleus; NM — nuclear membrane.

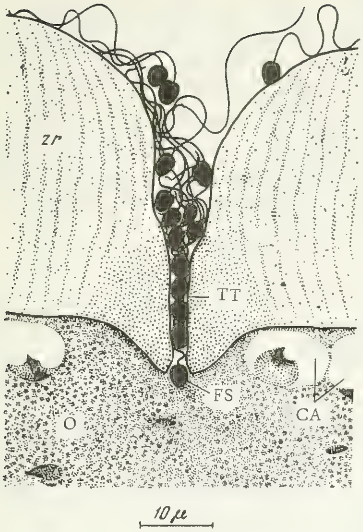

FIGURE 49. Egg of lake trout with spermatozoa deep in micropylar canal (insemination in cavity fluid or Ringer's solution; after Ginsburg, 1963b):

CA — cortical alveoli; TT — terminal tubule of micropyle; O — ooplasm; FS — fertilizing spermatozoon; zr — zona radiata.

It should be noted that Ballowitz (1904, 1913) correctly interpreted the function of the lamprey's acrosomal filament half a century before this was firmly established. He wrote that the "head bristle" penetrates the cytoplasm first, stimulates the cytoplasm to activity and then serves as a manubrium to draw the spermatozoon into the egg. Ballowitz indicated that the spermatozoon itself is passive in this process since it loses motility after penetrating the egg membranes.

The course of the second phase of gametic association differs substantially in sturgeons, since here the acrosomal reaction does not serve as a means for penetrating the membrane barrier. The spermatozoon comes into direct contact with the cortical cytoplasm deep in the micropylar canal. This contact, apparently with the participation of the acrosomal reaction, leads to immediate activation of the egg. Thus, 5 seconds after insemination of Husó huso, Acipenser güldenstädti and A. stellatus eggs, there are observable changes in the cortical layer near the micropyle, which are completed shortly after the contents of the cortical granules are discharged. At this time, the spermatozoon already is located in the micropylar canal, and afterward it gradually becomes absorbed by the cytoplasm (Ginzburg, 1957b, 1959).

The mechanism of gametic association in teleosts whose spermatozoon lacks an acrosome differs greatly from that described above. It would be extremely interesting to follow ultramicroscopically the morphology of this process in these fishes. Since the gametic association occurs here through direct fusion of the sperm head surface and the cortical layer, the role of the cell membranes must be particularly evident.

It should be noted that fertilization in teleosts, exactly as in sturgeons, requires calcium ions (Ginzburg, 1963b).

The gametic association is regularly accompanied by activation of the egg, but in salmonids these two processes may easily be separated experimentally and combined in arbitrary sequence (Ginzburg, 1963a, b).

When salmonid eggs are inseminated in cavity fluid or Ringer's solution they show no activation although the spermatozoon in the micropylar canal reaches the cortical layer and its head is partly immersed in the ooplasm (Figure 49). Nevertheless true sperm-egg association occurs, since such eggs develop exactly like eggs inseminated in water when the spermatozoa on their surface are first destroyed (Privol'nev, 1941) or after the spermatozoa lose their fertilizing capacity (Kusa, 1950). Zygotes with quiescent spermatozoa in the micropylar canal can be activated by water after intervals of several seconds, hours or days after insemination (Ginzburg, 1963a, b).

On the other hand, gametic association must occur within a given time after the eggs have been activated by water, as shown in chum salmon (Kusa, 1950) and trout (Ginzburg, 1963a, b). Gametic association was impossible from the moment cortical granules discharged into the micropylar region and blocked penetration of spermatozoa.

3.3. SPECIFIC EGG AND SPERM SUBSTANCES INFLUENCING GAMETIC ASSOCIATION

The eggs and the surrounding cavity fluid and the sperm contain substances having specific effects on both gametes.

In the 50 years since the investigations of Lillie (1913a, 1914, 1919) and Loeb (1914), these substances have been intensively studied.

Until recently no standard terminology was worked out for these substances. Lillie first drew attention to the biological function of the substances discharged by the gametes. As already mentioned, the substance discharged by eggs and causing agglutination of the spermatozoa was called fertilizin, while the name antifertilizin was given to the substance inactivating fertilizin. Even today, Tyler and his associates use the terminology based on Lillie's concepts. They identify 4 substances or groups of substances: fertilizin, antifertilizin of the spermatozoon, antifertilizin of the egg and lytic agents of the sperm. This cannot be accepted as a rational terminology since the significance of fertilizin in fertilization has not been confirmed. Moreover, new substances, specifically affecting the gametes and having no connection with fertilizin, are continually being discovered.

Hartmann and Kuhn (see Hartmann and Schartau, 1939; Hartmann, 1940) called the substances excreted by the gametes gamones (i.e., fertilization hormones or gametic hormones), which are divided into gynogamones and androgamones (products of eggs and spermatozoa, respectively). This terminology is widely used, but is also questionable since the analogy to hormones is debatable.

The terminology proposed by Dalcq (1928), which contains no preconceived ideas concerning the function and mechanism of the agent, is perhaps more successful. He proposed calling the egg secretion ovulin (accordingly, calling the sperm secretion, spermulin). However, since this terminology has not been widely accepted, it will not be used here.

Gamones have been found in various plants, algae, fungi (Hartmann, 1956), and in four animal phyla of animals — mollusks, annelids, echinoderms and chordates (including Cyclostomi and fishes). Considerable data concerning the character of the effect, localization, properties and chemical nature of the gamones have accumulated, but are in many cases contradictory and difficult to assess, since they were derived from different objects by varying methods. The brief characterization of gamones given below emphasizes data obtained for fishes.

For a more thorough discussion of the question the reader is referred to special reviews (Tyler, 1948, 1949, 1955; Bielig and Medem, 1949; Medem, 1949; Runnström, 1952; Runnström et al., 1959; Rothschild, 1956, 1958; Metz, 1957a; Wiese, 1961; Dorfman, 1963).

3.3.1. Agents from Eggs

The substance stimulating movement of spermatozoa (gynogamone I). In many animals, spermatozoan movement to intensified in the immediate vicinity of the egg. This effect is due to egg secretions in cavity fluid or egg water. In various kinds of marine invertebrates — sea urchins, mollusks, and annelids — egg water was found to increase the motility of the spermatozoa and prolong their active movement. In some cases, the increased motility is accompanied by increased oxygen consumption (Gray, 1928b; Tyler, 1948; Hathaway, 1963).

The eggs of vertebrates also can produce a substance activating spermatozoan movement. This was first established for the lamprey egg in whose immediate vicinity spermatozoa became more motile. This effect was demonstrated in egg water and in cavity fluid diluted with water (Schartau and Montalenti, 1941).

Eggs of Salmo irideus and S. trutta must also discharge gynogamone I, since spermatozoa move much more intensively and longer in cavity fluid than in water (Hartmann, 1944; Hartmann et al., 1947b). Prolongation of active movement of spermatozoa in cavity fluid was demonstrated for many fish species (see p. 138), but this is not always combined with increased motility. In sperm of 3 races of Coregonus lavaretus and of pike diluted with pure cavity fluid, only some spermatozoa were activated and their motility was reduced. However, in cavity fluid diluted by an equal amount of water, all spermatozoa are immediately activated but show no distinct increase in motility, although the total duration of the swimming period increases (Medem et al., 1949; Roth et al., 1950). The stimulating effect of cavity fluid on the spermatozoa is not universal in fishes; spermatozoa of Huso huso, Acipenser güldenstädti, A. stellatus, Alosa volgensis, loach, Diptychus, Schizothorax, Rutilus frisii kutum and Vimba vimba remain immotile in cavity fluid.

A clear example of the activating effect of gynogamone I is provided by fertilization in Clupea harengus pallasi (Yanagimachi and Kanoh, 1953; Yanagimachi, 1957c, e). Spermatozoa are almost immotile in seawater and move actively only if they come in contact with the surface of a mature egg near the micropyle. The activating substance is located in the egg membrane of the micropylar region and passes from there into the water. After removal of the membranes, the egg does not discharge this substance. Gynogamone I is rapidly destroyed by solutions of $CaCl_2$ and $MgCl_2$, acidified Ringer's solution, trypsin and heating to 60—70°C. After such inactivation, spermatozoa are not activated in the region of the micropyle; eggs, so treated, remain unfertilized on insemination, even though they can develop parthenogenetically. These experiments seem to indicate the requirement for gynogamone I in fertilization of Pacific herring eggs. However, under other experimental conditions spermatozoa penetrate the egg after removal of the egg membrane even in the absence of gynogamone I (Yanagimachi, 1957d).

Similar data were obtained (R. Suzuki, 1958, 1959b, 1961a, b) for the Japanese bitterlings Acheilognathus lanceolata, A. tabira and Rhodeus ocellatus and gudgeon Sarcocheilichthys variegatus. Spermatozoa of these fishes move energetically in water, and it is impossible to discern differences in the intensity of movement at different regions of the egg. However, when movement of the spermatozoa becomes slower, their increased activity near the micropyle becomes discernible and they concentrate there. The activating agent is not species specific; spermatozoa of both species accumulate near the micropyles of bitterling and Sarcocheilichthys eggs. A distinct, though weaker reaction is shown by spermatozoa of Cyprinus carpio and Carassius carrassius auratus.

In bitterlings, as in Pacific herring, gynogamone I is present in the egg membrane in the micropylar region and is not discharged by eggs lacking membranes. There is none inside the egg, since ooplasm extruded on heating of an eel egg does not effect activation and accumulation of spermatozoa.

Gynogamone I is rapidly removed from the membrane in an acid medium (R. Suzuki, 1960). Eggs treated with acidified Ringer's solution (pH 4.2) and inseminated with very dilute sperm (10^{-9}) show only 3–6% fertilization (against 79–86% fertilization in the control). If more concentrated sperm (10^{-5}) is used for insemination, the frequency of fertilization reaches 63–71%. Although not essential for the gametic association, gynogamone I seems to increase considerably the likelihood of the gametic encounter and, therefore, obviously has an important function in natural breeding conditions.

It has been suggested that gynogamone I not only activates spermatozoa, but also has a chemotactic effect, causing them to concentrate around the egg (aggregation). However, the chemotactic mechanism of spermatozoon attraction in animals still remains unknown. Such a mechanism has been proven for ferns, mosses, horsetails (Equisetaceae) and many other plants (Machlis and Rawitscher-Kunkel, 1963), but no conclusive data are available for animals. The concentration of spermatozoa in capillary tubes containing eggs or their secretion has repeatedly been described. Such experiments, however, are not conclusive, since the chemotactic effect cannot be differentiated from the effect of a "trap mechanism" (Rothschild, 1951, 1956; Tyler, 1955).

The gynogamone I molecule in sea urchins and fishes is relatively small and is dialyzable; in most of the species studied it showed considerable thermostability (Cornman, 1941; Kuhn and Wallenfels, 1940; Vasseur and Hagström, 1946; Hartmann et al., 1947a, b; R. Suzuki, 1960, 1961b; Hathaway, 1963). Thus, it can be regarded as distinct from the agglutinating factor (gynogamone II), which is a thermolabile and has a high molecular weight. Tyler (1955) suggests that the activating factor is usually closely associated with the agglutinating factor, but may be detached from it under certain conditions.

Hartmann and Kuhn and their coworkers provided data indicating that gynogamone I is a pigment: the naphthoquinone pigment echinochrome A in the sea urchin Arbacia pustulosa (Hartmann et al., 1939; Kuhn and Wallenfels, 1940) and the fat-soluble carotinoid astaxanthin ($C_{40}H_{52}O_4$) in trout (Hartmann et al., 1947a, b).

The identity of gynogamone I with echinochrome A in sea urchins was subsequently disproven (Tyler, 1939; Cornman, 1941; Bielig and Dohrn, 1950).

Data on the role of astaxanthin in the gametic association of fishes encounter a series of objections. Hartmann and others have not demonstrated whether astaxanthin is present in natural fertilization conditions or whether it is present in the cavity fluid of trout used for the biological investigation of gynogamone I. These authors indicated only that this fluid was yellowish, suggesting that it contained carotinoids. The color of the fluid would be more intense if it did in fact contain the concentrations of astaxanthin shown to be effective in experiments with crystalline pigment (Goodwin, 1952). Moreover, gynogamone I is known to be present in the egg membranes and absent in the ooplasm of Pacific herring (Yanagimachi, 1957e) and bitterling (R. Suzuki, 1958, 1961a). Little is known on the chemical properties of gynogamone I in these fishes, and available data are contradictory. This factor in bitterlings is thermostable (at 100–104°C for more than an hour) and not of protein nature, since it is unaffected by trypsin (R. Suzuki, 1960). Unlike all the other animals studied in this respect, gynogamone I in Pacific herring is thermolabile (inactivated at 70°C within 15 minutes) and is inactivated by trypsin, thus it has protein properties (Yanagimachi, 1957e).

Substances agglutinating homologous spermatozoa (also called isoagglutinins, gynogamone II and fertilizin). The eggs of many animals (echinoderms, mollusks, annelids, tunicates, lampreys, fishes, mammals) secrete substances which agglutinate spermatozoa. Initially, the spermatozoa adhere in small groups, which increase in size and form large agglomerates. Agglutinated spermatozoa retain motility and perform fluctuating movements. In sea urchins (Lillie, 1913a, 1941) and lampreys (Schartau and Montalenti, 1941) agglutination is reversible; a short while after being formed the lumps disperse and spermatozoa swim freely again. In other animals agglutination is irreversible (Tyler, 1948; Metz, 1957a).

In fishes — brook trout, grayling, whitefish, pike (Rötheli et al., 1950) and lake trout (Ginzburg, unpublished) — small agglomerates form in the first few seconds after the agglutinating agent is added to actively moving spermatozoa. The spermatozoa adhere by their heads and tails (in lake trout initially only by their tails). Such agglomerates enlarge with the attachment of new spermatozoa, uniting among themselves to form a wide-meshed network. At this time the spermatozoa still move quite energetically. When all movement ceases later, the cross-pieces of the net begin to break up, sections of the network contract and mass concentrations appear. Ultramicroscopic study of agglutinated spermatozoa (Rötheli et al., 1950) indicates that the heads adhere in dense masses, some of which swell strongly. The tail flagella form loops and show signs of cytolysis; the flagella contours become indistinct and the fibrils indiscernible, and rod-shaped formations about 0.001μ wide and $0.03-0.30\mu$ long appear one behind the other in place of the individual fibril.

In most invertebrates studied (see Tyler, 1948; Rothschild, 1956; Metz, 1957a), in lampreys (Schartau and Montalenti, 1941) and in Salmo irideus

(Hartmann et al., 1947a, b), agglutination occurs on addition of egg water to a suspension of spermatozoa. Moreover, in lamprey, Salmo irideus and Thymallus vulgaris (Roth et al., 1950), spermatozoa agglutinate in diluted cavity fluid. On the other hand, agglutinins are not present in egg water and cavity fluid in Salmo trutta m. fario and cavity fluid in Coregonus lavaretus and pike (Hartmann et al., 1947b; Roth et al., 1950).

The eggs are the source of agglutinins. In sea urchins the agglutinating agent enters the milieu from the jelly coat and, as has now been established, is identical with its substance (Tyler and Fox, 1940; Tyler, 1941, 1948; Vasseur, 1952). Loeb (1914) first indicated and many other investigators subsequently confirmed that unfertilized eggs do not secrete fertilizin after the jelly coat is removed. Chemically, the agglutinating substance is a compound of sulfonated saccharides and amino acids, apparently linked in a polypeptide chain, i.e., glycoproteins (Vasseur, 1948, 1952; Tyler, 1955; Metz, 1957a; Runnström et al., 1959); the molecular weight of agglutinin of Arbacia is about 300,000 (Tyler, 1956).

In addition to the agglutinin (or fertilizin) of the jelly coat, there is also an agglutinin, called cytofertilizin, within the sea urchin egg; this agglutinin is released from the egg after fertilization and is present in the perivitelline fluid (Motomura, 1950, 1953). Cytofertilizin may also be released by aging unfertilized eggs on dissolution and discharge of the cortical granules preceding general cytolysis. This led to the suggestion that cytofertilizin is localized in the cortical granules (Hagström, 1956c), which was confirmed by immunological methods. The surface of the fertilized sea urchin egg, i.e., the hyaline layer formed by cortical granules, reacts with rabbit fertilizin antiserum (Tyler and Brookbank, 1956), indicating the similarity of the reactive groups of the hyaline layer and fertilizin.

It should be noted that cytofertilizin differs in some properties from fertilizin of the jelly coat, i.e., the optimal pH of extraction (Motomura, 1950) and the effect on gametic association and formation of the fertilization membrane (Hagström, 1956c). This permits assuming that they also differ chemically.

In fishes the substance agglutinating spermatozoa (gynogamone II) is contained within the egg. Thus, it corresponds not to fertilizin but to the cytofertilizin of sea urchins.

If egg contents are removed from the membrane and dispersed in water, energetic agglutination of the spermatozoa is observed. Contents of one brook trout egg diluted in 11 liters of water were sufficient to cause agglutination (Hartmann et al., 1947b).

Gynogamone II was found in eggs of rainbow trout and grayling (in which it is discharged by unfertilized eggs into the milieu), as well as in brook trout, whitefish and pike eggs (which do not excrete it). The optimal concentration of egg contents, favoring strongest agglutination, is 2.2–4% in Coregonus lavaretus and 0.2–2% in pike; at higher concentrations agglutination is weak or absent. In brook trout and grayling, maximum agglutination is caused by a greater (50%) concentration of egg contents; the intensity of agglutination gradually diminishes according to the degree of dilution, but may still be considerable even at concentrations of 1% in grayling and 0.1% in trout (Medem et al., 1949; Roth et al., 1950; Rötheli et al., 1950).

Gynogamone II was liberated from rainbow trout eggs (Hartmann et al., 1957a, b). An active principle was obtained from the contents of unfertilized eggs by repeated precipitations in water and acetone, dissolution in 1% sodium chloride and dialysis. The dried preparation caused agglutination of spermatozoa in a solution of 5×10^{-9}.

The rainbow trout gynogamone II has a large molecule (nondialyzable); it is thermolabile (inactivated at 90°C in 2 minutes). Its properties indicate that it is a proteid consisting of a globulin-type protein and phosphatide. Thus, its chemical nature differs from the fertilizin of sea urchins which is a glycoprotein.

Gynogamone II from pike eggs is also thermolabile (inactivated at about 100°C within 2 minutes) (Roth et al., 1950).

I found an agglutinating substance in eggs of Salmo trutta m. lacustris which, like cytofertilizin of sea urchins, is discharged on fertilization and forms part of the perivitelline fluid in subsequent stages. Trout cytofertilizin, like that of sea urchins, is apparently located in the cortical bodies, whose contents form the perivitelline fluid (in this particular case, in the cortical alveoli). Addition of perivitelline fluid to a suspension of trout spermatozoa causes strong agglutination (Ginzburg, 1960b, 1961a; see Figure 50). The tail flagella of the spermatozoa become sticky and form loops, often surrounding the head in a characteristic way; tails of neighboring spermatozoa stick together. A study of the properties of perivitelline fluid collected from a large number of lake trout eggs showed that the agglutinating agent gives Millon and biuret reactions, is precipitated by sulfosalicyl acid and rapidly inactivated by trypsin and, consequently, is protein in nature. Unlike the gynogamone II preparation from unfertilized rainbow trout eggs, this agent has considerable thermostability (boiling for 90—100 minutes is required for inactivation). This difference does not yet allow the conclusion that the agglutinins of lake and rainbow trout are two different substances, since gynogamone II may possibly undergo transformations during activation of the egg.

Spermatozoa of mammals (rabbits, mice, bulls), like those of the animals described above, are agglutinated in the presence of eggs or their extracts (Bishop and Tyler, 1956).

The agglutinins of the different animal species are specific. However, there are known cases of "heteroagglutination" in which the gynogamone II of one species exerts an agglutinating effect on spermatozoa of another species and sometimes even of another phylum (Lillie, 1913a; Hartmann et al., 1940; Tyler, 1948; Bielig and Medem, 1949; Rothschild, 1956).

The function of agglutinins in the gametic association has been debated for many years and is still not fully elucidated. We have already mentioned Lillie's fertilizin theory of fertilization and Tyler's theory of specific pinocytosis, each of which regard the gametic association as resulting from specific interaction between the fertilizin of the egg and the antifertilizin of the spermatozon. However, the basic assumption of these theories that fertilizin is essential for fertilization has not been confirmed. Actually, Lillie's fertilizin which proved to be identical with the jelly coat of the sea urchin egg cannot directly participate in the gametic association because of its location. Experimental removal of the jelly coat (and, consequently, removal of fertilizin) proved that fertilizin is not essential for fertilization,

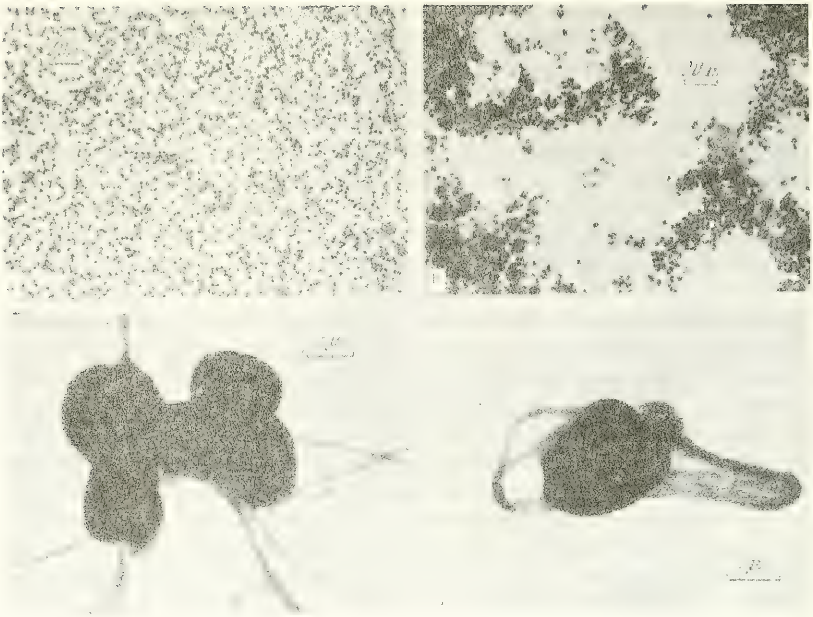

FIGURE 50. Agglutination of lake trout spermatozoa on addition of perivitelline fluid:

a — sperm suspended in water; b — sperm suspended in perivitelline fluid — vigorous agglutination; c, d — electron micrograph of spermatozoa agglutinated in perivitelline fluid-water (1:4)

since such eggs remained fertile. Tyler countered this argument with his own data (Tyler, 1941, 1948) that eggs without a jelly coat require a higher concentration of spermatozoa for their fertilization than intact eggs, indicating that the presence of fertilizin promotes fertilization. However, later it was shown that these results were due to damage of the eggs on removal of the jelly coat; if this coat is removed carefully, fertilization of all the eggs requires a much lower spermatozoan concentration, and gametic association occurs more rapidly than in the control (Hagström, 1956a, b). Hagström's numerous experiments indicate that the jelly coat acts as a sieve removing part of the spermatozoa, and is an important factor protecting the egg from polyspermy (which will be discussed in more detail in Chapter 6). Moreover, this membrane acts as a barrier protecting the egg from penetration of alien spermatozoa (Hagström, 1956a, b, d, e, f; Runnström et al., 1959).

How does the jelly coat material effect elimination of spermatozoa? Reversible agglutination is observed in sea urchin spermatozoa suspended in egg water or a solution of the jelly coat (fertilizin). Although the spermatozoa retain their motility, the fertilizing capacity of the suspension is sharply reduced (Lillie, 1913a; Tyler, 1941; Hagström, 1959a; Metz, 1961). Supporters of the fertilizin theory explain this fact, which directly indicates the adverse effect of fertilizin on spermatozoa, as saturation of reactive groups on the spermatozoan surface by complementary groups of fertilizin molecules, resulting in the loss of the capacity for interaction with the egg fertilizin. A better founded explanation was given by Dan (1954b), who showed that spermatozoa are reversibly agglutinated, but no acrosomal reaction can take place in egg water almost completely devoid of calcium ions. In this case, the fertilizing capacity of the suspension remains unchanged. In ordinary egg water containing calcium, most of the spermatozoa eject an acrosomal filament after agglutination ceases, and the fertilizing capacity of the suspension is thus sharply reduced. This naturally leads to the conclusion that the loss of fertilizing capacity is not connected with agglutination of the spermatozoa, but with the occurrence of an acrosomal reaction. In light of the recent data, it has become accepted that a spermatozoon which has undergone an acrosomal reaction at a distance from the egg loses the means for penetrating the vitelline membrane, despite remaining motile.

With regard to agglutinins contained in the egg (cytofertilizin of sea urchins and the agglutinating agent in fish eggs), these substances, apparently located in the cortical granules and alveoli, could not possibly fulfill a direct function in the gametic association. When the fertilizing spermatozoon contacts the egg and activates it, these substances are discharged under the membrane, and create a protective layer inhibiting penetration of supernumerary spermatozoa into the cytoplasm (see Chapters 5 and 6).

Thus, agglutinins do not link the spermatozoon and the egg, as suggested by the fertilizin theory and its successors until recently, but serve as a means for protecting the egg from polyspermy.

A substance inactivating the agglutinating agent (antifertilizin of eggs). Sea urchin eggs contain a substance, released upon cytolysis, which inactivates fertilizin and is named antifertilizin (Lillie, 1914). Antifertilizin precipitates jelly coat material and the egg agglutinating agent. Egg antifertilizin is protein in nature and similar in its properties to antifertilizin of the spermatozoa (Tyler, 1940). Antifertilizin may be excreted both from unfertilized and fertilized sea urchin eggs. It is not clear whether it plays any role in fertilization (Tyler, 1948; Metz, 1957a, b; Runnström et al., 1959).

A substance inducing the acrosomal reaction. The group of specific substances contained in the female sexual products apparently must also include a chemical agent inducing the acrosomal reaction, which is excreted into the milieu by unfertilized eggs of many invertebrates, lampreys and sturgeons. No data on its properties and chemical nature are available, but the possibility cannot be excluded that it is gynogamone II. This suggestion is supported by the occurrence in certain cases of a strong agglutinating effect of egg water and its capacity for inducing ejection of acrosomal filaments in a high percent of spermatozoa (Metz and Morril, 1955). In the absence of calcium ions, however, it is possible to separate the agglutination reaction and the ejection of acrosomal filaments (Dan, 1954b).

3.3.2. Agents from Sperm

A substance suppressing spermatozoan motility (androgamone I). A substance arresting the movement of spermatozoa has been extracted from spermatozoa of sea urchins, mollusks, salmonids and some other animals. This substance, named androgamone I, is an antagonist of gynogamone I. In the sea urchin Arbacia pustulosa it neutralizes the spermatozoan-activating effect of gynogamone I, while a preparation of gynogamone I can eliminate its immobilizing effect (Hartmann et al., 1940). The same applies to the preparations from rainbow trout. Addition of androgamone I to boiled cavity fluid containing gynogamone I or to a solution of gynogamone I weakens the activating effect and even abolishes it at increased amounts; on the other hand, gynogamone I restores motility to spermatozoa immobilized by androgamone I (Hartmann et al., 1947b).

The active agent, extracted from spermatozoa of sea urchins and salmonids, is soluble in methanol, dialyzable, thermostable and not sensitive to trypsin; consequently, it is not a protein. It can affect spermatozoa of other species, orders or even phyla (Hartmann et al., 1940, 1947; Runnström et al., 1944; Rothschild, 1951).

It is not certain whether this agent is released to the milieu from the spermatozoa under physiological conditions. Hartmann and his coworkers (Hartmann et al., 1940, 1947) attributed the immotility of the spermatozoa in the seminal fluid to the presence of androgamone I. But this was not confirmed in the case of sea urchins in which immotility is apparently due to oxygen deficiency (Rothschild, 1948a). However, spermatozoa of salmids, unlike those of the sea urchin, are not activated when the oxygen concentration is increased, indicating that the seminal fluid of salmon actually contains immobilizing substance (Runnström et al., 1944; Rothschild, 1951, 1958). This is proved indirectly by the observations that the supernatant (which may contain the active agent of the spermatozoa) of heat-coagulated sperm of rainbow and brook trout (Hartmann et al., 1947) and untreated seminal fluid of trout, whitefish, grayling and pike (Medem et al., 1949; Roth et al., 1950) immobilize spermatozoa and neutralize gynogamone I.

Very little is known concerning the chemical nature of the immobilizing agent from the fish seminal fluid. In rainbow trout it is most likely an inorganic substance, since the reconstituted ash of dried seminal fluid in distilled water inhibits movement of the spermatozoa. This same effect is exerted by a solution of inorganic salts prepared at concentrations corresponding to those in the seminal fluid of trout. However, if potassium chloride is replaced by sodium chloride in the solution, the immobilizing effect is lost. This suggests that the active principle in the seminal fluid of trout is potassium (Schlenk and Kahmann, 1938).

A substance neutralizing the agglutinating agent (androgamone II, antifertilizin of spermatozoa). A methanol-insoluble substance extracted by various methods from sea urchin spermatozoa was found to resemble the antifertilizin of eggs in neutralizing gynogamone II, precipitating the jelly coat matter and agglutinating the eggs (Frank, 1939; Tyler, 1940). This substance is an acid protein of molecular weight less than 10,000 (Runnström et al., 1942; also see Tyler, 1948, Metz, 1957a). Salmon spermatozoa also excreted a protein substance, insoluble in methanol, which precipitates the gel of the jelly coat of sea urchin eggs (Runnström et al., 1944). A similar spermatozoan preparation from rainbow trout causes the membrane of its own eggs to become turbid, perhaps analogous to the gel precipitation of the sea urchin jelly coat.

The substance inactivating gynogamone II is present not only in the spermatozoa but also in the seminal plasma of lampreys (Schartau and Montalenti, 1941) and of several fishes — whitefish (Medem et al., 1949), pike and grayling (Roth et al., 1950). No changes were effected in the egg membranes of whitefish and pike by their own seminal plasma.

Substances dissolving egg membranes and the cortical layer (lysins of the spermatozoon). Lysins of egg membranes were found in the sperm of mollusks, annelids, echinoderms, enteropneusts (see Tyler, 1948; Rothschild, 1956; Metz, 1957a; Wiese, 1961; Dorfman, 1962, 1963), lampreys (Kille, 1960a) and amphibians (Hibbard, 1928a, b; Wintrebert, 1929). Hyaluronidase of mammals should also be numbered among substances of this type (Austin and Bishop, 1957a; Chang, 1957; Dorfman, 1962, 1963). There are no corresponding data for fishes in the literature.

The lysin of egg membranes of Mytilus edulis is a thermolabile protein (Berg, 1950). The lysin of the jelly coat of eggs of the sea urchin Mellita quinquiesperforata (Brookbank, 1958) is also highly thermolabile (inactivated at 70°C within 2 minutes), rapidly broken down in an acid medium and clearly distinguished in its properties from the antifertilizin of the spermatozoon. Using a purified preparation, the agent dissolving the jelly coat of eggs of the sea urchin Anthocidaris crassispina was shown to be a species-specific enzyme (Isaka et al., 1966).

The earlier idea (Hibbard, 1928b; Dan, 1952) that the lysin of the spermatozoon is located in the acrosome was later confirmed experimentally. After centrifugation of inactivated spermatozoa of Mytilus, the supernatant showed no lytic activity and the lysin could be extracted from the spermatozoan precipitate. In spermatozoa which have undergone the acrosomal reaction (caused by addition of excess calcium), the greater part of the lysin is found in the supernatant (Wada et al., 1956). The liberated lysin dissolves the hyaline layer of the vitelline membrane, but has no effect on the jelly coat (Dan, 1962). This feature completely conforms with observations of gametic association in bivalve mollusks in which, as in sea urchins, the spermatozoa swim through the jelly coat and are stopped only at the vitelline membrane where the acrosomal reaction occurs (Dan and Wada, 1955).

Spermatozoa of the annelid Hydroides hexagonus from which the lytic agent has been extracted by freezing-thawing technique have an opening in the acrosomal vesicle (Colwin and Colwin, 1961a). Openings in the acrosomal vesicle also appear when the spermatozoon penetrates the vitelline membrane and the acrosomal granule in the vesicle dissolves and disappears within a few seconds. Dissolution of the granule is accompanied by lysis of the section of the vitelline membrane adjacent to the acrosome, leading to the conclusion that the lysin originates in it (Colwin and Colwin, 1961b).

The location of the lysin of the egg membrane in the acrosome was also confirmed for mammals. Spermatozoa of the mouse (Ohnuki, 1959) and rabbit (Moricard, 1960; Moricard et al., 1960; Hadek, 1963a) lose the acrosomes on passing through the transparent egg membrane; the acrosomes are always absent in spermatozoa which have penetrated the perivitelline space. Moreover, a cylindrical "tunnel", 2 μ in diameter, is left in the wake of a rabbit spermatozoon advancing into the zona pellucida, which ends in a cone before

TABLE 27. Comparison of the gamones of sea urchins and teleosts (for references see text)

	Active agent	Sea urchins			Teleosts		
		biological effect	location	chemical characteristics	biological effect	location	chemical characteristics
Agents from eggs	The substance stimulating movement of spermatozoa (gynogamone I)	Increases motility of spermatozoa and, in some species, oxygen consumption; prolongs period of motility; antagonist of androgamone I	In egg, excreted into egg water	Low-molecular weight, thermostable substance. Not identical with echinochrome A	Increases motility of spermatozoa and prolongs period of motility; antagonist of androgamone I	In Salmo egg, excreted into cavity fluid and egg water; found in cavity fluid in Coregonus, Thymallus and Esox; in micropylar region of egg membranes of Acheilognathus and Clupea (absent in egg)	Agent from Salmo is considerably thermostable, but is destroyed on exposure to light, and, supposedly, is the carotinoid astaxanthin; a thermostable substance of a non-protein nature in Acheilognathus and a thermolabile protein in Clupea
	Substances agglutinating homologous spermatozoa (isoagglutinin, gynogamone II) a) fertilizin	a) Causes reversible agglutination of spermatozoa; antagonist of androgamone II	a) Identical to material of jelly coat of egg, liberated into egg water	A glycoprotein containing sulfonated saccharides. Molecular weight in Arbacia punctulata about 300,000. Thermolabile, possessing acid properties			
	b) cytofertilizin	b) Causes agglutination of spermatozoa	b) In egg (apparently cortical granules), secreted into perivitelline space		b) Causes irreversible agglutination of spermatozoa; antagonist of androgamone II	b) In egg. In Salmo irideus and Thymallus vulgaris liberated into cavity fluid, in S. irideus also into egg water; not excreted by unfertilized eggs in	In Salmo irideus a proteid consisting of globulin type protein and phosphatide. Thermolabile

						S. trutta m. fario, Coregonus lavaretus and Esox lucius. In S. trutta m. lacustris apparently located in cortical alveoli and liberated into perivitelline space	
	Substance inactivating the agglutinating agent (antifertilizin of eggs)	Precipitates gel of jelly coat, causes agglutination of eggs; antagonist of gynogamone II	In egg	Protein			
Agents for sperm	A substance suppressing spermatozoan motility (androgamone I)	Immobilizes spermatozoa; antagonist of gynogamone I	In spermatozoa (In seminal fluid)	Low-molecular weight, thermostable substance, soluble in methanol and resistent to trypsin (Immobilizing effect due to lack of oxygen and not androgamone I)	Immobilizes spermatozoa; antagonist of gynogamone I	In spermatozoa of Salmo In seminal fluid of Salmo, Coregonus, Esox and Thymallus	Low-molecular weight, thermostable substance, soluble in methanol and resistent to trypsin (Immobilizing effect in seminal fluid of S. irideus due to inorganic substance, possibly potassium ions)
	A substance neutralizing the agglutinating agent (antifertilizin of spermatozoa, androgamone II)	Precipitates gel of jelly coat, causes agglutination of eggs; antagonist to gynogamone II	In spermatozoa	Relatively thermostable protein with acid properties. Molecular weight less than 10,000	Causes turbidity of chorion in trout eggs and precipitates gel of jelly coat in sea urchin eggs; antagonist of gynogamone II	In spermatozoa of Salmo, in seminal fluid of Salmo, Coregonus, Esox, Thymallus	Relatively thermostable, protein, not soluble in methanol

TABLE 27 (Continued)

	Active agent	Sea urchins			Teleosts		
		biological effect	location	chemical characteristics	biological effect	location	chemical characteristics
Agents from sperm	Substances dissolving egg membranes (lysins of the spermatozoon)	Dissolves jelly coat and vitelline membrane of egg	Lysin dissolving vitelline membrane apparently located in acrosomal granule of spermatozoon	Lysin dissolving jelly coat is a thermolabile protein rapidly broken down in an acid medium			
	Substance liquidizing the cortical layer (androgamone III)	Dissolves or liquidizes surface layer of cytoplasm	In spermatozoa	Low-molecular weight, thermostable compound, apparently an unsaturated fatty acid			

the sperm head (Moricard, 1960; Moricard et al., 1960). Thus, disappearance of the acrosome is accompanied by excretion of a lysin dissolving the membrane.

It may be assumed that spermatozoa of fishes whose egg membranes lack micropyles (selachians and chimaeras) contain lysins which dissolve the membrane, just like those in spermatozoa of lampreys, amphibians and other animals with a similar kind of gametic association; however, no investigations have been made on such animals.

In addition to the lysin dissolving the egg membranes, another type of lysin found in sea urchins liquidizes the cortical layer. This lysin, named androgamone III, is extracted from spermatozoa by methanol, has low-molecular weight and is thermostable with surface-active properties. It is apparently an unsaturated fatty acid (Runnström et al., 1944; Tyler, 1948).

The data given above concerning the biological effect, location and chemical nature of fish gamones have been summarized in Table 27. These are compared with data on gamones of sea urchins, since this group is most completely known in this respect and for other aspects of the early embryonic stages.

3.3.3. Biological Role of Substances Participating in Gametic Association

The importance of the substances just considered was much overrated for many years. Lillie and his followers considered the agglutinating agent (fertilizin) as an essential link between the spermatozoon and the egg. There is now every reason to reject this hypothesis. The function of the gamones was somewhat exaggerated and simplified by Hartmann, who considered the success of fertilization dependent on the interaction of balanced quantities of androgamones and gynogamones (Hartmann et al., 1940; Hartmann, 1956). It seems most correct to regard these substances as ancilliary agents of the fertilization process, facilitating the gametic encounter and association. Many such substances are probably still unknown.

The egg secretion (gynogamone I), which activates and prolongs movement of the spermatozoa, accelerates and increases the probability of contact between the gametes. An agent liberated by the egg, which causes the acrosomal reaction, has not yet been identified. Lysins of the sperm dissolve egg membranes and assist in overcoming the barrier between the spermatozoon and the egg. The substance of the seminal plasma immobilizing spermatozoa aids in prolonging retention of fertilizing capacity, but does not participate directly in the gametic association. The role of the factors which agglutinate the sperm remain most controversial. The best-founded view seems to be that the agglutinin of the jelly coat of sea urchins, etc. (fertilizin) acts as a sieve to eliminate part of the spermatozoa even before contact is established between the gametes. With regard to the agglutinin contained in the egg of sea urchins, sturgeons and teleosts (cytofertilizin), the main function seems to me to be protection of the fertilized egg from supernumerary spermatozoa (see Chapter 6). The role of certain other substances discharged from gametes – antifertilizin of the eggs and spermatozoa, substances liquidizing the cortical layer of the egg – is still unclear.

3.4. CONCENTRATION OF SPERMATOZOA NECESSARY FOR FERTILIZATION

A considerable concentration of spermatozoa is required to achieve maximum fertilization, i.e., for each fertilizable egg to become fertilized. If insemination is performed by an insufficiently dense suspension of spermatozoa, some eggs remain unfertilized because the spermatozoa lose their motility before contacting all the eggs. In some cases where spermatozoa long retain motility, the percent of fertilization decreases because the eggs lose their fertility before reached by the spermatozoa.

With sturgeons and salmonids, we performed special experiments to clarify the concentration of spermatozoa giving a maximum percentage of fertilization. For this purpose aliquots of roe from a single female, each containing several hundred eggs, were inseminated with the milt of a single male which was diluted variously with water immediately before insemination.

In 4 experiments on Huso huso, 11 on Acipenser güldenstädti and 1 on A. stellatus, a maximal percentage of fertilization was achieved with a suspension containing 10^5 spermatozoa/cc (which corresponds to dilution of the ejaculate in water in the ratio of 1:10,000). In 2 experiments on A. güldenstädti and 3 on A. stellatus this result was obtained only with a more concentrated suspension containing 10^6 spermatozoa/cc (dilution 1:10,000). The results of several of these experiments are shown in Figure 51, curves 1—3.

It should be noted that these results, obtained in the laboratory with small portions of roe and milt known to be of good quality, cannot be obtained in fishery practice without modifications. In the case of commercial insemination of large quantities of sturgeon roe (several kilograms at a time), it is necessary to use a more concentrated milt suspension to obtain maximum fertilization percentage (Ginzburg et al., 1963; Ginzburg, 1963; see Supplement).

Insemination of lake trout eggs with diluted sperm requires a higher concentration of spermatozoa than in the case of sturgeons (Figure 51, curves 4 and 5). The maximum percentage of fertilization is obtained in suspensions of 10^7—10^9 spermatozoa/cc (1:1,000—1:9 dilution in water) and only exceptionally by less concentrated suspensions of 10^6 spermatozoa/cc. This is probably due to the much shorter lifespan of salmonid spermatozoa in water compared to those of sturgeons (see Table 24). A lower concentration is sufficient when lake trout eggs are inseminated in cavity fluid where spermatozoa retain their motility much longer.

In considering the concentration of spermatozoa necessary for maximum fertilization, it should be remembered that the number of spermatozoa activated by

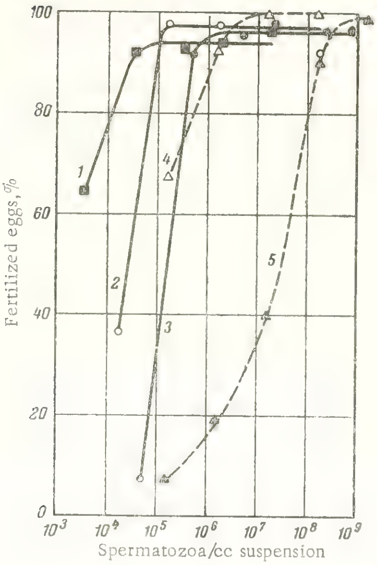

FIGURE 51. Concentration of spermatozoa ensuring maximal fertilization percentage for Acipenser güldenstädti colchicus (1), Huso huso (2), A. stellatus (3) and lake trout (4, 5)

water is important and not the absolute concentration. In addition, the properties (motility, duration of swimming in water, resistance to external influences) of spermatozoa of the individual male are important.

A decrease in the fertilization percent is also observed when the dilution in water is too low. In experiments on Huso huso and Acipenser güldenstädti, sperm diluted in an equal volume of water gave a decreased fertilization (compared to experiments with less concentrated suspensions) of 12–13%, while a dilution of 10 parts sperm in one part water gave 46% less fertilization. This deterioration in insemination efficiency with poorly diluted suspensions of spermatozoa is apparently due to their reduced activity in such conditions ("dilution effect", see p. 127).

3.5. TIME REQUIRED FOR GAMETIC ENCOUNTER

When eggs are inseminated with suspensions containing hundreds of thousands or millions of spermatozoa per cc, a considerable number of spermatozoa must be close to the egg at any one time. Their further approach to the egg depends on random movements in chance directions. A certain time is required for the movement of the spermatozoon to end in the establishment of contact with the cortical surface.

This length of time can be determined experimentally by interrupting the interaction time at different periods by destroying the spermatozoa without damaging the eggs. Sea urchin spermatozoa were inactivated by hypotonic seawater (Rothschild and Swann, 1959) and the detergent sodium lauryl sulfonate (Hagström and Hagström, 1954a). To inactivate sturgeon spermatozoa T. A. Detlaf used 0.1 and 0.2 M sodium chloride solutions (unpublished data, see Ginzburg, 1957b). This method has the disadvantage that complete inactivation of the spermatozoa is not achieved at once, and isolated eggs were fertilized for 15 seconds after the addition of the salt solution.

In my experiments on Acipenser stellatus, A. güldenstädti (Ginzburg, 1957b) and lake trout a 0.5% solution of the detergent "Novost'" of the Kazan Fats Combine (containing sulfoesters of cetyl and stearyl alcohol) caused instantaneous cessation of movement of spermatozoa, followed by lysis (in dilute suspensions of spermatozoa, complete lysis occurs with 20–30 seconds). Eggs of Acipenser stellatus, A. güldenstädti or trout taken from the body cavity were placed in short glass tubing covered at one end with loose silk cloth. The tube with the eggs was immersed in the freshly diluted sperm and was shaken continuously to wash the eggs in the sperm suspension. After varying lengths of time, the tube was transferred to the detergent suspension for inactivation of the spermatozoa, thereby precisely limiting the sperm-egg interaction time. The eggs were then transferred to water and left to develop to the second division stage. Those eggs contacted by spermatozoa before transfer to the detergent became fertilized and showed cleavage, while the other eggs remained unchanged. A total of 12 experiments were performed on sturgeons (10 with eggs of Acipenser stellatus and 2 with eggs of A. güldenstädti) and 6 on lake trout. Results of 4 experiments are shown in Figure 52. Sperm-egg interaction for only 1–2 seconds can lead to a considerable proportion of fertilization. The time required for all fertilizable eggs to make contact with spermatozoa is 3—6 seconds in trout, 10—15 seconds in Acipenser stellatus and A. güldenstädti.

Trout spermatozoa are not only activated by water but also by cavity fluid. To test the sperm-egg interaction time in cavity fluid it was necessary to change the procedure, since the amount of cavity fluid available was limited. A portion of trout eggs with a small amount of cavity fluid was placed in a Petri dish, inseminated with drops of "dry" sperm, and mixed with a feather. After a certain time "Novost'" solution was poured into the dish. A small percentage of eggs were fertilized in the first few seconds; 33.3% were fertilized within 4 seconds, 73.7% within 8 seconds and 85.2% in 10 seconds (100% fertilization in the control). These figures are somewhat low, since it is impossible to mix the eggs and sperm as well as when shaking a tube of eggs in an aqueous suspension of sperm. Apparently the time necessary for sperm-egg interaction in cavity fluid is similar to that required for their association in water.

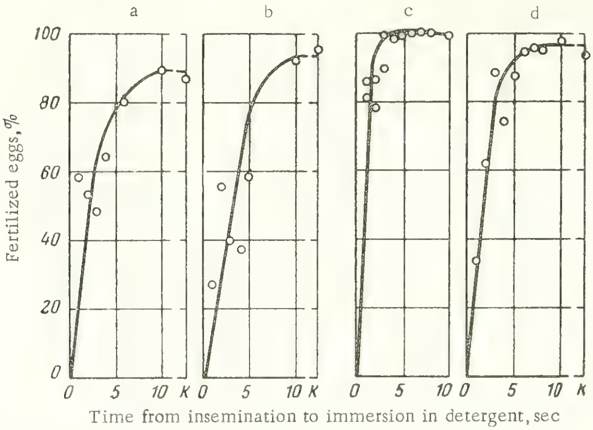

FIGURE 52. Time required for sperm-egg contact in Acipenser stellatus (a, b) and lake trout (c, d).

Concentration of spermatozoa per cc suspension: a — 5.9×10^7; b — 8.4×10^7; c — 1.2×10^8; d — 3.7×10^6.

These time periods are of the same order as those established for sea urchins. All Psammechinus miliaris eggs were fertilized in less than 5 seconds with a concentration of about 10^7 spermatozoa/cc and at a temperature of 16—18°C (Rothschild and Swann, 1952). Fertilization of Strongylocentrotus droebachiensis eggs at the same spermatozoan concentration and a temperature of 8°C required 5—10 seconds (Ginzburg, 1963). When a less concentrated suspension of spermatozoa is used, the time required for fertilization of all the eggs is greatly prolonged. It is 45 seconds in P. miliaris with a concentration of about 10^5 spermatozoa/cc (Rothschild and Swann, 1951), 20—30 seconds in S. droebachiensis with a concentration of about 10^6 spermatozoa/cc and 60 seconds or more with a concentration of about 10^5 (Ginzburg, 1963c).

The time required for the gametic encounter is similar in fishes and in sea urchins; it may differ substantially in other animals having the same method of insemination. A sharply divergent case apparently is that of bivalve mollusks, which are of particular interest here because of their special mechanism for blocking polyspermy. In experiments with Ostrea gigas, sodium lauryl sulfonate was used to inactivate the spermatozoa (after Hagström and Hagström, 1954a). Even when concentrated suspension of spermatozoa (about 10^7/cc) are used, only a very small proportion of the eggs become fertilized in the first few minutes after insemination. About 20 minutes are required to obtain maximum fertilization percentage (Ginzburg, unpublished).

The data obtained for fishes indicate that the duration of spermatozoan activity is quite sufficient for fertilization of all the eggs, even where it is extremely brief and forward movements, as in most salmonids, last only 20–60 seconds (see Table 24).

Chapter 4

NUMBER OF SPERMATOZOA PENETRATING EGG AND FATE OF SUPERNUMERARY SPERMATOZOA

Karyogamy, i. e., fusion of male and female pronuclei takes place after gametic association. Invariably only one male pronucleus participates in karyogamy, although the egg of many animals is regularly penetrated by several spermatozoa.

Two types of fertilization are distinguished with respect to the number of spermatozoa normally penetrating the egg: monospermy and physiological polyspermy.

In monospermic fertilization, only one spermatozoon enters the egg which is adapted to block the entrance of supernumerary spermatozoa into the ooplasm (block to polyspermy). However, in certain cases the block to polyspermy may be ineffective, for example, in artificial insemination with highly concentrated sperm or under physiological conditions unfavorable to the eggs. Then, the ooplasm is penetrated by several spermatozoa which participate in development, disrupting morphogenesis so that eggs soon die (pathological polyspermy).

Physiological polyspermic eggs are usually penetrated by several or even many spermatozoa. Only one male pronucleus participates in formation of a synkaryon; the remainder are excluded from karyogamy and are soon eliminated through special adaptations absent in monospermic eggs. Thus, the embryo develops without deviations from the normal (provided that the intensity of polyspermy does not exceed certain species-specific limits).

The occurrence of polyspermy in the animal kingdom and its forms have been considered by Godlewski (1913), Wilson (1925) and many others. Rothschild (1956) devoted attention to the mechanism of the block to polyspermy. A short survey of polyspermy from the viewpoint of Michurin biology was given by Lebedev (1963). A recent summary of physiological and pathological polyspermies in animals by Piko (1961) is excellent, but deals only with data on birds and mammals.

This chapter will deal with results of investigations on the number of spermatozoa penetrating the egg and their subsequent fate in various groups of fishes. Corresponding data for other animals will also be considered, in order to give a complete picture of polyspermy, its distribution, consequences and correlations with egg structure (described in Chapter 1), conditions of the gametic encounter and type of fertilization. Information on the number of spermatozoa penetrating the egg in various animals is summarized in Tables 28 and 29.

4.1. SELACHIANS

Fertilization is internal in all selachians, both viviparous and oviparous, and evidently occurs before the egg is enclosed in a dense sperm-impermeable capsule. The gametic encounter is widely considered to take place in the upper part of the oviduct above the shell gland.

No spermatozoa were found in Scyliorhinus caniculus and Mustela canis in this section of the oviduct and in the anterior part of the shell gland secreting the albumin, but were invariably encountered in the lumen of the tubules of the median and posterior sections of the shell gland in all females at various seasons (Metten, 1939; TeWinkel, 1956). This fact indicates that the shell gland acts as a receptaculum seminis. Metten observed release of spermatozoa on secretion of the capsule material, and concluded that fertilization in selachians takes place directly in the shell gland together with formation of the capsule after the egg is surrounded by albumin. However, this conclusion, based on indirect data, is insufficiently founded. In particular, it is incompatible with Rückert's (1899) observations that in Pristiurus melanostomus spermatozoa are located in the ooplasm at the entrance to the shell gland (when only part of the egg extended into it). In such eggs spermatozoa had already begun to change into pronuclei, indicating that fertilization occurred either directly before entry to the shell gland or even earlier in the adjoining part of the oviduct.

It was already shown at the end of the last century that the germinal disc of selachian eggs is penetrated by a considerable number of spermatozoa.

Kashchenko (1888a, b, 1894) found a large number of nuclei in eggs of Scyliorhinus caniculus and Pristiurus melanostomus soon after fertilization. Up to 25 nuclei of similar structure and size were found in the germinal disc of eggs removed from the shell gland. At a somewhat later stage when the eggs had just emerged from the shell gland, there was one large nucleus in the center of the germinal disc while the rest had shifted to the periphery. Although the patterns of polyspermic fertilization of selachian eggs were first described by Kashchenko, he was unable to assess correctly these patterns, since he did not have material on a series of consecutive stages of fertilization. He therefore suggested that the numerous nuclei in uncleft eggs result from divisions of the egg before the appearance of furrows.

Soon after Kashchenko's first reports, Rückert established that nuclei present in the blastodisc of fertilized selachian eggs before cleavage are not derivatives of the zygotic nucleus, but appear even before fusion of the male and female pronuclei as a result of transformation of the sperm heads (Rückert, 1891a, b, 1892b, 1899, 1910). The results of investigations of fertilization and cleavage in selachian eggs were dealt with exhaustively by Rückert in a general monograph in 1899. The basic objects of the study were Pristiurus melanostomus and Torpedo ocellata (also Scyliorhinus caniculus for cleavage stages).

Rückert showed that the selachian egg is regularly penetrated by several or many spermatozoa. There was no case of monospermy among 109 eggs at fertilization stages and the first cleavage division. The number of spermatozoa penetrating varies from 2 and 7 to 47 spermatozoa in the germinal disc of P. melanostomus (in most cases 10–30) and from 1 and 4 to 56 in Torpedo ocellata (10–30 in most eggs). The surface of the germinal disc is depressed where the spermatozoon penetrates, then gradually smoothes. Spermatozoa enter the egg both in the central and in peripheral parts of the disc, as well as in the surrounding zone of finely granular yolk. In Torpedo, moreover, many spermatozoa penetrate in the area of the coarsely granular yolk. Many spermatozoa, in certain eggs considerably more than 100, were found in the cytoplasmic bond surrounding the germinal disc and slightly wider in diameter than the disc. In Pristiurus no spermatozoa were found in this region.

Penetration of spermatozoa into the blastodisc is not strictly simultaneous, as indicated by the following data. The number of spermatozoa in two Pristiurus blastodiscs fixed at the beginning of fertilization (when sperm heads change into pronuclei) was only 3 and 8, whereas blastodiscs fixed at the end of this period generally contained far more spermatozoa. The structure of the heads in the two youngest blastodiscs differed, since they were at different stages of transformation. However, these differences among spermatozoa in a single blastodisc were small and soon disappeared (even before fusion of the pronuclei), indicating the very short time period in which spermatozoa enter the ooplasm. In this respect, fertilization in selachians apparently differs from that of chimaeras and reptiles (see below).

From Rückert's investigations it is clear that all the spermatozoa entering the blastodisc change in a similar manner; the heads become spherical, swell and are transformed into pronuclei of similar size and structure. Concentrations of cytoplasm of the germinal disc form around the pronuclei, bounded by lighter sections (Figure 53, a). One of the male pronuclei, apparently the closest to the female pronucleus fuses with it (this occurs while the egg is still in the shell gland). The remaining male pronuclei are distributed evenly in the germinal disc (Figure 53, b), according to Rückert, due to a repulsive effect on one another (or rather, of the seminal asters accompanying them).

During the first two mitotic cleavage divisions the sperm pronuclei grow synchronously with the synkaryon and its derivatives or at a very slight delay. These pronuclei show regular bipolar mitosis, differing from division of the synkaryon in small spindle size and haploid number of chromosomes. Derivatives of the synkaryon occupy the central part of the germinal disc and gradually push the nuclei derived from the supernumerary spermatozoa to the periphery and even beyond to the yolk-rich cytoplasm. In Torpedo this usually occurs at the end of the second or beginning of the third division (Figure 53, c), in selachians (Pristiurus and Scyliorhinus caniculus) the period of displacement of the male pronuclei is prolonged.

When the central part of the germinal disc is divided by the first furrows, superficial furrows are often found at the periphery, partially dividing small areas of cytoplasm containing nuclei derived from supernumerary spermatozoa (Figure 53, d, e). This phenomenon was noted already by Kashchenko (1888b, 1894). Only exceptionally do such peripheral blastomerelike formations become completely separated from the yolk. Additional furrows do not appear in all eggs, and these disappear in the later cleavage stages (see, for example, Figure 9, a).

While in the germinal disc, the nuclei originating from supernumerary male pronuclei differ from cleavage nuclei only in their small size. After

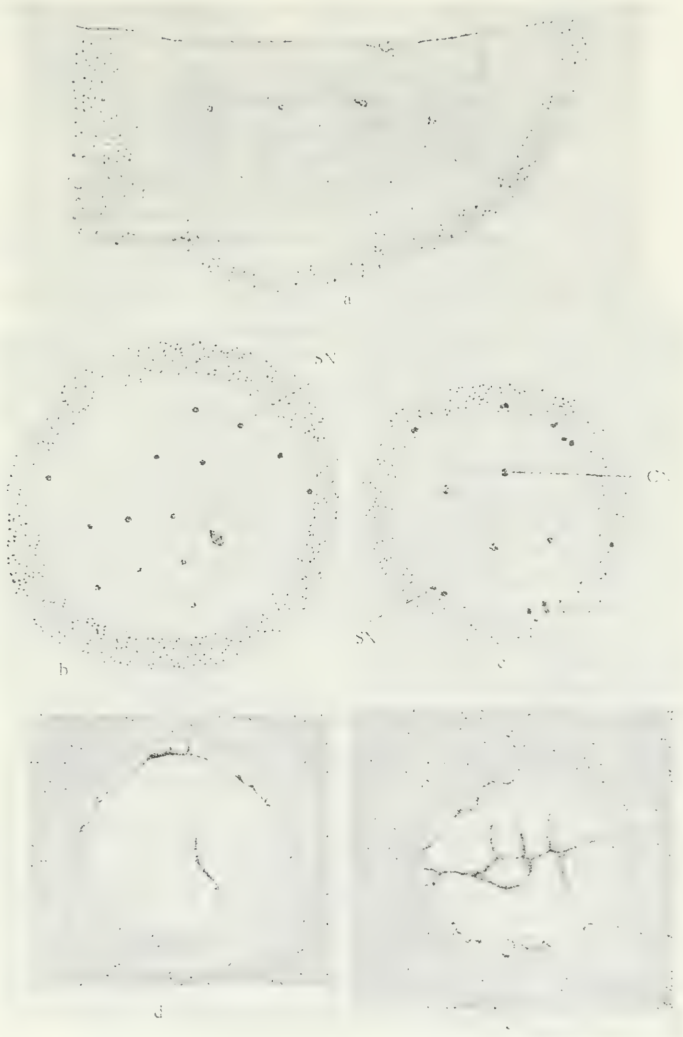

FIGURE 53. Fertilization and first cleavage stages in selachians:

a — cross section of germinal disc at stage of pronuclei fusion, with polar body at surface; b — stage of first cleavage division; c — stage of 4 cleavage nuclei; d, e — superficial furrows at periphery of germinal disc (with 1 or several cleavage furrows) separating sections of cytoplasm with supernumerary male pronuclei; a, e — Pristiurus melanostomus, b, c, d — Torpedo ocellata (after Rückert, 1899). SN — supernumerary male pronuclei; CN — cleavage nucleus.

displacement into the yolk-rich region of the egg, they display a number of characteristics which become more and more pronounced as the egg develops. The divisions begin to lag behind those of the cleavage nuclei, and are most retarded the farther the nuclei are from the boundary of the germinal disc. The daughter nuclei often remain close together, so that groups of 2, 4 and 8 nuclei are formed. Breaches occur in the mitotic spindle structure and in the distribution of the chromosomes in metaphase. Where the nuclei are particularly close, multipolar mitosis and accompanying irregular distribution of chromosomes among the daughter nuclei occur, leading to great variability in the size of the interkinetic nuclei.

Despite these various deviations, the nuclei originating from the supernumerary spermatozoa retain typical metaphase structure (degenerated nuclei are rare) for a long period, (at least until the blastula stage) and continue to divide. According to Rückert's data, they are transformed into nuclei of vitelline syncytium (periblast, parablast, according to the terminology of various authors, or nuclei of merocytes of Rückert).

Derivatives of the supernumerary spermatozoa do not participate in formation of the embryo. This was proven, on the one hand, by a study of consecutive developmental stages which traced step-by-step the displacement of the supernumerary nuclei from the germinal disc, and, on the other hand, by Rückert's calculation of the number of blastomeres in the disc, which always corresponds to that of each particular cleavage stage. Consequently, the derivatives of the supernumerary spermatozoa do not remain in the germinal disc (otherwise the number of blastomeres or nuclei would be greater than expected) and the derivatives of the synkaryon at these stages do not participate in forming yolk nuclei (otherwise the number of blastomeres would be less than expected). Furthermore, Rückert does not exclude the possibility that part of the nuclei in the vitelline syncytium are derived at later stages from another source, at the expense of nuclei of the peripheral blastomeres, as occurs in teleosts, reptiles and birds.

Supernumerary spermatozoa which penetrate from the very beginning in the zone of coarsely granular yolk degenerate rapidly, and do not form nuclei capable of division.

These data indicate that fertilization in selachians is physiological polyspermic. The germinal disc is regularly penetrated by a considerable number of spermatozoa, which undergo similar changes at first. Then, the male pronuclei (except for the one fusing with the female pronucleus) are displaced from the germinal disc; these remain viable for a long period but do not participate in the formation of the embryo.

4.2. CHIMAERAS

Fertilization is also polyspermic in chimaeras, which resemble selachians in egg structure and conditions of insemination (Dean, 1903, 1906). In Hydrolagus (Chimaera) colliei, many spermatozoa — more than twenty — enter the blastodisc. As in selachians, small depressions in the surface remain at the points of spermatozoan penetration into the egg. The spermatozoa penetrate at various points of the blastodisc, both centrally and at the periphery. The depression becomes exceedingly small immediately after the sperm head is embedded in the ooplasm, but gradually increases in diameter as the head advances into the blastodisc. The varying sizes of depressions on the blastodisc surface indicate the considerable duration of penetration of the spermatozoa. This is confirmed by study of sections of eggs. At the stage of karyogamy, recently penetrated sperm heads are visible alongside supernumerary male pronuclei. Subsequent movement and behavior of the supernumerary male pronuclei are apparently similar in chimaeras and selachians, but were studied much less fully in chimaeras.

4.3. CHONDROSTEAN FISHES

In sturgeons, in contrast to selachians and chimaeras, the gametic encounter occurs in the water outside the female. The egg membranes of these fishes are impermeable to spermatozoa, which reach the ooplasm only through the micropylar canals. However, since egg membranes of sturgeons are provided with several such canals (from 1—2 to 40 or more, see Table 9), several spermatozoa actually could penetrate the egg at once.

Cytological data on fertilized eggs of Acipenser ruthenus seem to indicate that physiological polyspermy is inherent in sturgeons, as in selachians and chimaeras (Persov, 1954a, 1956, 1957). Sections of several eggs contain considerable spermatozoa (from 2 to 28) in the ooplasm. Supernumerary spermatozoa were found from several minutes after insemination to long after formation of pronuclei and their fusion. The supernumerary sperm heads retained their original shape at all times tested. Spermatozoa were located at various (sometimes considerable) depths of the ooplasm; some of them were found at the boundary of the animal and vegetal poles, others in the vegetal pole among large clumps of yolk. During cleavage, multipolar mitoses characteristic of pathological polyspermy were found. On this basis, Persov concluded that polyspermy in Acipenser ruthenus is physiological.

However, more thorough cytological investigations under various conditions of insemination and comparisons of cytological patterns with observed egg development showed that the sturgeons have monospermic fertilization. Polyspermy may be obtained experimentally, but it is distinctly pathological (Ginzburg, 1953, 1957a, c; 1959, Ginzburg et al., 1963).

To study fertilization in Huso huso (Ginzburg, 1959), a portion of eggs was inseminated with strongly diluted sperm (dilution in water 1:10,000; concentration of spermatozoa in the suspension, 4.59×10^5/cc). Sections of 397 eggs were studied cytologically, and in every case, without exception, only one spermatozoon had penetrated the cytoplasm and only one male pronucleus was found in later stages.

The same result was obtained when eggs of Acipenser güldenstädti were inseminated with equally dilute sperm (Ginzburg, 1957a). However, when a more concentrated sperm suspension was used (1:100), two cases (8%) among 25 eggs showed two male pronuclei each; 20 (74%) eggs out of 27 proved to be polyspermic with an even denser sperm suspension (1:9· concentration of spermatozoa, 1.89×10^9/cc).

TABLE 28. Number of spermatozoa penetrating egg of animals with external fertilization

Phylum, class, order	Species	Number of spermatozoa penetrating egg	Author
Cnidaria			
Hydrozoa			
Leptolida	Pennaria tiarella	Usually monospermy, but occasionally two or more spermatozoa penetrate (artificial insemination)	Hargitt, 1909
Nemertini			
Nemertini			
Heteronemertini	Cerebratulus marginatus	Monospermy; dispermic eggs develop abnormally	Coe, 1899
	Cerebratulus leidyi		
	Micrura caeca		
Annelida			
Polychaeta			
Errantia	Nereis limbata	Monospermy	Lillie, 1911, 1912, 1913a
	Ophryotrocha puerilis	Monospermy; development atypical in cases of polyspermic fertilization	Korschelt, 1895
Sedentaria	Chaetopterus pergamentaceus	Monospermy; polyspermic eggs encountered rarely	Mead, 1895, 1898
	Arenicola marina	Monospermy	Child, 1898
	Pectinaria gouldii	Monospermy; multipolar mitotic figures in case of rarely observed polyspermy	Austin, 1953
	Sabellaria alveolata	Monospermy	Fauré-Fremiet, 1924
	Pomatoceros triqueter		Monroy, 1948
Clitellata			
Oligochaeta	Rhynchelmis limosella	Monospermy; occasionally egg penetrated by 2–6 spermatozoa	Vejdovsky, 1888–1892
	Eisenia (Allolobophora) foetida	Usually monospermy; sometimes 2 or more spermatozoa (up to 9)	Foot, 1894, 1898
		Development normal at low degree of polyspermy	Devriès, 1964a
Echiurida	Thalassema mellita	Monospermy	Griffin, 1899
Echiurinea	Ikedosoma gogoshimense		Sawada and Ochi, 1962

Arthropoda			
Crustacea			
Amphipoda	Gammarus duebeni	Monospermy	Le Roux, 1933
	Orchestia gammarella		Campbell-Parmentier, 1963
Mollusca			
Bivalvia			
Filibranchia	Mytilus edulis	Monospermy	Meves, 1915
Eulamellibranchiata	Unio complanata	Monospermy; polyspermy rare exception	Lillie, 1901
	Barnea spinosa (B. candida)	Monospermy	Pasteels, 1961, 1965a
	Zirfaea crispata		Griffin, 1899
Echinodermata			
Asteroidea			
Cryptozonia	Asterias glacialis	Monospermy; polyspermic eggs develop abnormally	Fol, 1877 b, c, d, 1877–1879
	Asterias forbesei	Monospermy; dispermic eggs develop abnormally	Wilson and Matthews, 1895
Echinoidea			
Centrechinoida	Arbacia punctulata	Monospermy	Just, 1939
	Paracentrotus (Toxopneustes) lividus	Monospermy; polyspermic eggs develop abnormally	Hertwig, 1875; Fol, 1877a, d, 1877–1879; Flemming, 1882
	Sphaerechinus brevispinosus		Fol, 1877a, d, 1877–1879; Flemming, 1882
	Lytechinus (Toxopneustes) variegatus		Wilson and Matthews, 1895
	Psammechinus (Echinus) miliaris		Fleming, 1882
	Psammechinus (Echinus) microtuberculatus		Boveri, 1908
	Paracentrotus (Strongylocentrotus) lividus		Boveri, 1908
Chordata			
Enteropneusta	Saccoglossus kowalevskii	Monospermy	Colwin and Colwin, 1953, 1954b
Ascidiacea			
Enterogona	Ciona intestinalis	Monospermy	Boveri, 1890; Castle, 1896; Conklin, 1905
		Monospermy; polyspermic eggs occasionally encountered	Gólski, 1899
	Phallusia mammilata	Monospermy	Hill, 1895

TABLE 28 (Continued)

Phylum, class, order	Species	Number of spermatozoa penetrating egg	Author
Pleurogona	Styela partita	Monospermy; dispermic eggs, incapable of development, occasionally encountered	Conklin, 1905
Cephalochordata	Branchiostoma (Amphioxus) lanceolatus	Normally monospermy; multipolar mitotic figure in case of polyspermy	O. Van der Stricht; 1895; Sobotta, 1895b; 1897
Cyclostomi			
Petromyzoniformes	Lampetra fluviatilis	Monospermy	Kupffer and Benecke, 1878; Herfort, 1901; Kille, 1960a
		Monospermy; polyspermy very rare	Chubareva, 1957a, b
	Lampetra planeri	Monospermy	Calberla, 1878; Boehm, 1888; Kille, 1960a
	Lampetra japonica		T. S. Yamamoto, 1956
Pisces			
Acipenseriformes	Huso huso	Monospermy	Ginzburg, 1959
	Acipenser ruthenus	Usually monospermy; sometimes in fertilized eggs from 2 to 28 unchanged spermatozoa encountered	Persov, 1954a, 1956, 1957
	Acipenser güldenstädti	Monospermy; polyspermic eggs develop abnormally	Ginzburg, 1953, 1957a , b;
	Acipenser stellatus		Ginzburg et al., 1963
Clupeiformes	Clupea harengus pallasi		Yanagimachi and Kanoh, 1953; Kanoh, 1953
	Oncorhynchus keta	Monospermy	K. Yamamoto, 1952a; Kanoh, 1957
	Salmo trutta m. lacustris		Ginzburg, unpublished
	Salmo trutta m. fario		Boehm, 1892; Behrens, 1898
	Salmo irideus		Behrens, 1898
Cypriniformes	Acheilognathus lanceolata	Monospermy	R. Suzuki, 1959a
	Rhodeus ocellatus		
	Carassius auratus	Monospermy	Tchou-Su and Chen-Chao-hsi, 1936; Ozima, 1943
	Cyprinus carpio		
Cyprinodontiformes	Oryzias latipes	Monospermy	Sakai, 1961
Perciformes	Crenilabrus pavo	Monospermy	Hoffmann, 1881
	Julis vulgaris		
	Scorpaena scrofa		
	Scorpaena porcus		

Pleuronectiformes	Limanda schrenki	Monospermy	K. Yamamoto, 1952b
Amphibia			
Caudata	Hynobius nebulosus	Monospermy	Kunitomo, 1910; Nakamura, 1933; Kakiyama, 1934
	Hynobius retardatus	Generally monospermy	Makino, 1934
	Cryptobranchus alleghaniensis	Occasional polyspermy, artificially inseminated eggs taken from oviducts	Smith, 1912
Salienlia	Discoglossus pictus	Monospermy	Wintrebert, 1933
	Pelobates fuscus	Monospermy; polyspermic eggs die soon	Born, 1886; 1892
	Bufo lentiginosus	Monospermy in all cases	King, 1901
	Bufo cinereus	Monospermy	Born, 1886
	Rana temporaria (R. fusca)	Monospermy; polyspermic eggs develop abnormally	Hertwig, 1877; Roux, 1887; A. Brachet, 1910a, b; 1912; Herlant, 1911
	Rana arvalis	Monospermy	Born, 1886
	Rana esculenta		Hertwig, 1877; Born, 1886; Roux, 1887

In the latter batch, it was possible to trace how several (up to 4) spermatozoa simultaneously penetrate each of the many eggs through different micropyles (Plate IX, a). Heads of spermatozoa which penetrated change synchronously; first they form compact nuclei of a specific shape (Plate IX, b), then increase in size, acquire a vesicular structure and are drawn deep into the cytoplasm of the animal pole (Plate IX, c), each leaving a distinct spermatic trace. While male and female pronuclei approach and fuse in monospermic eggs, in polyspermic eggs all the male pronuclei generally aggregate close the female pronucleus to form a compact group (Plate X, a). This group is surrounded by a considerable number of cytocentra (Plate X, b); since each seminal aster divides into two cytocentra, the number of male pronuclei participating in fusion is usually doubled.

In the prophase of the first cleavage division, the membranes of the fusing pronuclei dissolve and numerous spindle figures appear, as is characteristic for pathological polyspermy in other animals (see below). There is a more isolated spindle, occasionally found in the vicinity of the central mitotic figure; this male pronucleus divides independently of the synkaryon. Somewhat later when furrows of the first cleavage division appear, the cytoplasm of the animal pole in polyspermic eggs divides directly into 3, 4 or more blastomeres (Figure 54, c—e). However, 2 blastomeres are formed in many dispermic eggs, as in monospermic eggs; at the second division two furrows appear in each blastomere, and the animal pole divides into 6 blastomeres (Figure 54, b, b'). New furrows appear from the beginning of the third and subsequent divisions in polyspermic eggs, so that they continue to differ from monospermic eggs in having an excessive amount of blastomeres (Ginzburg, 1953).

Formation of excessive blastomeres in polyspermic eggs at both second and

TABLE 29. Number of spermatozoa penetrating eggs of animals with internal fertilization

Phylum, class, order	Species	Number of spermatozoa penetrating egg	Author
Porifera			
Porifera			
Calcarea	Grantia compressa	Monospermy	Gatenby, 1920; Duboscq and Tuzet, 1937
	Sycon raphanus	Monospermy	Maas, 1899
		Monospermy; eggs with 3 pronuclei occasionally encountered	Duboscq and Tuzet, 1937
Cnidaria			
Hydrozoa			
Leptolida	Tubularia mesembryanthemum	Usually monospermy; polyspermic eggs sometimes encountered	Tikhomirov, 1887; Brauer, 1891b; Oschmann, 1923
		Usually monospermic eggs, but always polyspermic in derivatives of giant oocytes	Benoit, 1925
	Myriothela cocksi	Monospermy	Benoit, 1925
	Obelia (Gonothyrea) loveni	Usually monospermy; polyspermic eggs occasionally encountered	Wulfert, 1902
Hydrida	Hydra vulgaris (H. grisea)	Monospermy	Bauer, 1891a
Anthozoa			
Alcyonaria	Sympodium coralloides	Monospermy	Tuzet, 1941
Plathelminthes			
Turbellaria			
Polyclada	Eustylochus ellipticus	Monospermy; penetration by more than one spermatozoon extremely rare	Van Name, 1899
	Planocera nebulosa		
	Leptoplana tremellaris	Monospermy	Francotte, 1897
	Prostheceraeus vittatus		Klinckowström, 1897; Francotte, 1897
	Prostiostomum siphunculus		Francotte, 1898
	Cycloporus papillosus		
Triclada	Dugesia lugubris	Monospermy	Benazzi Lentati, 1962
	Dugesia benazzii	Polyspermy frequent in biotype P_2 which reproduces by pseudogamy	Benazzi Lentati, 1953

Protriclada	Olomesostoma auditivum	Monospermy	Hofsten, 1907, 1909; Reisinger, 1958
Eulecithophora	Mesostoma ehrenbergi		Bresslau, 1904
Archoophora	Polychoerus caudatus		Gardiner, 1898
Trematoda			
Strigeatoidea	Proterometra macrostoma	Monospermy	Anderson, 1935
Echinostomida	Fasciola hepatica	Monospermy	Henneguy, 1906; Schelleberg, 1911; Yosufzai, 1953
	Fasciola hepatica	Normal monospermic eggs; dispermy encountered in eggs not surrounded by shell	Bogomolova, 1956
	Parorchis acanthus	Monospermy	Rees, 1939
	Zygocotyle lunata		Willey and Godman, 1951
	Gigantocotyle bathycotyle		Willmott, 1950
	Macravestibulum kepneri		Jones et al., 1945
Plagiorchiida	Dicrocoelium lanceatum	Monospermy	Goldschmidt, 1908
	Collyriclum		Tyzzer, 1918
	Pneumonoeces (Haematoloechus) medioplexus		Burton, 1960
	Asymphylodora sp.		Dhingra, 1955
	Probolitrema californiense		Markell, 1943
	Gorgoderina attenuata		Wiley and Koulish, 1950
	Zoogonus mirus	Monospermy; polyspermic eggs occasionally encountered	Goldschmidt, 1905; Wassermann, 1913
Opisthorchiida	Cryptocotyle lingua	Monospermy	Cable, 1931
	Isoparorchis eurytremum		Dhingra, 1954
Monogenoidea			
Gyrodactyloidea	Gyrodactylus elegans	Monospermy	Gille, 1914
Polystomatoidea	Polystoma integerrimum		Goldschmidt, 1902; Halkin, 1902
Cestoda			
Pseudophyllidea	Diphyllobothrium latum	Frequent polyspermy (penetration by 2–3 spermatozoa)	Pavlova, 1955
Nemathelminthes			
Nematoda			
Nematodes	Parascaris equorum (Ascaris megalocephala)	Monospermy; 2 spermatozoa very rarely penetrate	Van Beneden, 1883; Boveri, 1888

TABLE 29 (Continued)

Phylum, class, order	Species	Number of spermatozoa penetrating egg	Author
		Dispermic eggs develop atypically	Boveri and Stevens, 1904
		Monospermy; very rarely 2–3 or more (up to 10) spermatozoa penetrate	Zacharias, 1887
		Monospermy; occasionally 2 or more (up to 8) spermatozoa penetrate	Makarov, 1953b; 1957a, b, 1964
	Toxocara canis (*Ascaris marginata*)	Monospermy; occasionally 2 spermatozoa penetrate	Kul'chitskii, 1888
	Rhabditis aberrans	Monospermy (pseudogamic reproduction)	Krüger, 1913
	Rhabditis monohystera	Monospermy	Belar, 1924
Annelida			
Polychaeta			
Errantia	*Platynereis megalops*	Monospermy	Just, 1939
Archiannelida	*Saccocirrus papillocercus*		Buchner, 1914
	Dinophilus apatris		Nachtsheim, 1919
Myzostomida	*Myzostomum parasiticum*	Monospermy;	Wheeler, 1895, 1898
	(*Myzostoma glabrum*)	Monospermy; dispermic eggs are rare exceptions	Kostanecki, 1898
Clitellata			
Hirudinea	*Erpobdella* (*Nephelis*) *Hemiclepsis* (*Clepsine*) *marginata*	Monospermy	Hertwig, 1877 Whitman, 1878
Arthropoda			
Arachnida			
Araneae	*Theridion tepidariorum*	1–4 spermatozoa; polyspermy rather frequent	Montgomery, 1907
Crustacea			
Branchiopoda	*Artemia salina*	Polyspermic eggs encountered	Fautrez-Firlefyn, 1951
Insecta			
Isoptera	*Kalotermes flavicollis*	1–3 spermatozoa; about 10% polyspermic eggs	Truckenbrodt, 1964
Homoptera	*Pseudococcus citri*	1–2 spermatozoa; 3% dispermic eggs	Schrader, 1923
	Pseudococcus obscurus	1–2 spermatozoa; about 4% dispermic eggs	Nur, 1962

Hemiptera	Pyrilla perpusilla	Monospermy	Sander, 1956
	Phyrrhocoris apterus	Several spermatozoa always penetrate, but only 1–3 male pronuclei (2–3 in 50% of eggs) are formed	Henking, 1892
Coleoptera	Agelastica alni	Several spermatozoa penetrate, but only 1–3 male pronuclei are formed; 16% polyspermic eggs	Henking, 1892
	Donacia (sericea?)	1–2 male pronuclei	Henking, 1892
	Adimonia tanaceti	1–2 spermatozoa transformed (polyspermy frequent)	Henking, 1892
	Tenebrio molitor	Polyspermy apparently frequent	Saling, 1907; Ewest, 1936
	Lytta viridana	Monospermy	Rempel and Church, 1965
Lepidoptera	Bombyx mori	1–2 spermatozoa	Henking, 1892
		1–11 spermatozoa; 85% polyspermic eggs	Kawaguchi, 1926
		Usually 1–3 spermatozoa, rarely up to 7	Bataillon and Tchou-Su, 1928, 1933; Tchou-Su, 1953
	Parum colligata	1–8 spermatozoa, usually 2–3; 75% polyspermic eggs	Tchou-Su and Chang-Ko, 1951
	Lymantria dispar	Frequently 2 spermatozoa	Seiler, 1914
	Lymantria japonica		
	Lymantria monacha		
	Phragmotobia fuliginosa	Usually 1 spermatozoon, occasionally 2 or sometimes 3	Seiler, 1914
	Diacrisia virginica	Monospermy	Johannsen, 1929
Hymenoptera	Lasius niger	Several spermatozoa frequently penetrate, but rarely more than 1 (2–3) male pronucleus is formed	Henking, 1892
	Apis mellifera	1–2 or more spermatozoa	Blochmann, 1889
		Often 2–3 spermatozoa, sometimes up to 7	Petrunkevich, 1901
		Usually 3–7 spermatozoa, rarely 1–2 and 8–10	Nachtsheim, 1913
	Platygaster dryomiae	Monospermy	Silvestri, 1916
	Paracopidosomopsis		Patterson, 1917
	Copidosoma gelechiae		Leiby, 1922
	Habrobracon juglandis	1–2 spermatozoa; 1% dispermic eggs	Speicher, 1936

TABLE 29 (Continued)

Phylum, class, order	Species	Number of spermatozoa penetrating egg	Author
Diptera	Drosophila melanogaster	From 1 to more than 30 spermatozoa; 97% polyspermic eggs	Huettner, 1924
		Usually about 12 or more spermatozoa	Huettner, 1927
		Usually 5–8 spermatozoa	Sonnenblick, 1950
		Usually 5–6 spermatozoa, rarely more than 10	Counce, 1959b
		1–2 spermatozoa; 5% dispermic eggs	Hildreth and Lucchesi, 1963
	Drosophila virilis	Monospermy	Hildreth and Lucchesi, 1963
	Calliphora (Musca) vomitoria	Usually 1 spermatozoon, sometimes 2–3	Blochmann, 1887
	Cochliomyia hominovorax	1–5 spermatozoa, mostly 2; 65% polyspermic eggs	Riemann, 1965
	Sciara coprophila	Usually 1 spermatozoon	Schmuck and Metz, 1932
	Sciara impatiens	Monospermy	Carson, 1946
	Dacus tryoni		D. T. Anderson, 1962
	Heteropesa pygmaea	1–3 spermatozoa, mostly 2	Camenzind, 1966
Mollusca			
Gastropoda			
Monotocardia	Lacuna vincta	Usually 1, sometimes 2 spermatozoa; invariably only 1 male pronucleus is formed	Kiknadze, 1956
	Crepidula plana	As a rule, monospermy; 2 spermatozoa are rare	Conklin, 1902
Pleurocoela	Cymbulia peronii	Monospermy	Nekrasov, 1904, 1909
Basommatophora	Physa fontinalis	Usually monospermy, but polyspermic eggs encountered	Kostanecki and Wierzejski, 1896; Wierzejski, 1905
	Lymnea elodes	Usually monospermy, but dispermic eggs encountered	Linville, 1900
	Lymnea stagnalis	Polyspermy as a rule; supernumerary spermatozoa degenerate rapidly, and only 1 male pronucleus is formed	Crabb, 1927; Bretschneider, 1946, 1948
		1–3 spermatozoa; not less than 54% polyspermic eggs	Horstmann, 1955

	Lymnea stagnalis Lymnea palustris Lymnea auricularia Bulinus (Isidora) contortus	1 male pronucleus; 2 male pronuclei rare	Larambergue, 1930, 1939
Stylommatophora	Arion empiricorum	Usually monospermy; sometimes egg contains unchanged supernumerary spermatozoa	Platner, 1886
		Monospermy	Lams, 1910; Perrot, 1939
	Limax agrestis	Usually monospermy; occasionally several (up to 12) spermatozoa penetrate	Byrnes, 1899
	Limax agrestis Limax maximum	Usually monospermy	Linville, 1900
	Eulota (Eulotella) similaris	Monospermy	Ikeda, 1930
	Helix aspersa	Usually monospermy; occasionally 2–3 spermatozoa penetrate, but only 1 male pronucleus is formed	Garnault, 1888–1889
	Helix pomatia	Usually monospermy, but often 4–5 spermatozoa penetrate; apparently only 1 male pronucleus is formed	Perrot, 1937
Bryozoa Ectoprocta Phylactolaemata	Lophopus cristallinus	Monospermy	Marcus, 1934
Eurystomata	Membranipora pilosa	Several dozen unchanged spermatozoa found in eggs	Bonnevie, 1907
		Normally monospermic	Just, 1934
Chaetognatha Chaetognatha	Sagitta bipunctata Sagitta serratodentata	Monospermy	Elpat'evskii, 1910, 1913
	Spadella cephalopterus	Monospermy	Vasiljev, 1925
Chordata Pisces Selachiformes	Pristiurus melanostomus	Several spermatozoa	Kashchenko, 1888a, b, 1894
		From 8 to 48 spermatozoa in germinal disc	Rückert, 1899

TABLE 29 (Continued)

Phylum, class, order	Species	Number of spermatozoa penetrating egg	Author
	Scyliorhinus caniculus (= *Scyllium canicula*)	Several spermatozoa (up to 25)	Kashchenko, 1888a, b, 1894
	Torpedo ocellata	From 2 to 57 (usually 10–30) spermatozoa in germinal disc; many spermatozoa also penetrate outside disc	Rückert, 1899
Chimaeriformes	*Hydrolagus* (*Chimaera*) *colliei*	Many spermatozoa (not less than 24)	Dean, 1906
Amphibia			
Caudata	*Ambystoma jeffersonianum*	5–8 spermatozoa	Piersol, 1929
	Ambystoma maculatum	5–8 spermatozoa	Piersol, 1919
	Ambystoma tigrinum (*Siredon mexicanum*, *Siredon axolotl*)	From 1 to 12 spermatic traces	Van Bambeke, 1870
		1–9 spermatozoa; polyspermy very frequent	Fick, 1893
		2–12 spermatozoa	Smith (after Piersol, 1929)
		From 1 to 9 spermatozoa (average 2.3); 48% polyspermic eggs	Sládeček and Lanzová, 1959
		From 1 to 15 spermatozoa (average 4.4); 75% polyspermic eggs	Pott, 1967
	Triturus vulgaris (*Triton taeniatus*)	1–3 or more spermatozoa; almost all eggs polyspermic	Michaelis, 1897
	Triturus (*Triton*) *alpestris*	3–9 spermatozoa	Braus, 1895
		From 1 to 10 spermatozoa, usually more than 3	Bataillon and Tchou-Su, 1929
	Triturus (*Triton*) *cristatus*	1–3 or more spermatozoa; almost all eggs polyspermic	Michaelis, 1897
		Intensity of polyspermy higher than in *Triturus alpestris*	Bataillon and Tchou-Su, 1929
	Triturus palmatus	From 1 to 9 spermatozoa, usually 2–4; 92% polyspermic eggs	Fankhauser, 1932
	Diemictylus (*Triturus*) *viridescens*	From 2 to 14 spermatozoa, usually 7–9; 100% polyspermic eggs	Jordan, 1893

		From 1 to 13 spermatic traces, usually 4–8	Kaylor, 1937
		From 1 to 19 spermatozoa, usually 2–6 (average 5.5); 94% polyspermic eggs	Fänkhauser and Moore, 1941a
	Triturus pyrrhogaster	From 1 to 32 spermatozoa, usually 1–9; 78% polyspermic eggs	Street, 1940
	Triturus pyrrhogaster subsp. orientalis (= Cynops orientalis)	From 1 to 16 spermatozoa (average 4.9); 73% polyspermic eggs	C. Chang et al., 1960
Reptilia			
Sauria	Anguis fragilis	From 1 to 6 spermatozoa (average 2.7); 67% polyspermic eggs	Oppel, 1892
		From 19 to 45 spermatozoa (average 25.8)	Nicolas, 1900
		From 1 to 5 spermatozoa	Nicolas, 1903
Serpentes	Natrix (Tropidonotus) natrix	From 10 to 38 spermatozoa (average 17.9)	Oppel, 1891
	Vipera (Pelias) berus	From 9 to 37 spermatozoa	Ballowitz, 1903b
Aves			
Galliformes	Gallus gallus	Usually 6–7 spermatozoa, exceptionally 25	Patterson, 1910
		3–4 spermatozoa	Olsen, 1942
		From 1 to 29 spermatozoa	Bekhtina, 1966
Columbae	Columba livia	From 13 to 26 spermatozoa	Harper, 1904
Mammalia			
Monotremata	Tachyglossus (Echidna) setosus	Monospermy (1 egg studied at pronuclei stage)	Flynn, 1930
		Monospermy (6 eggs studied at pronuclei stage and 6 at early cleavage stages)	Flynn and Hill, 1939
Chiroptera	Vesperugo noctula	Monospermy (not one polyspermic egg among 120 studied at pronuclei stage)	O. Van der Stricht, 1910
Glires	Cavia porcellus	Monospermy (1 degenerating polyspermic egg – 3.3% – out of 30 at fertilization stage)	Lams, 1913
	Rattus rattus	Monospermy (10 dispermic eggs – 1.2% – out of 810 fertilized eggs)	Austin and Braden, 1953
		Monospermpy (5 dispermic eggs – 1.8% – out of 284)	Austin, 1956b
		Monospermy (1 dispermic egg – 0.3% – out of 334)	Odor and Blandau, 1956
		Monospermy (0–3.2% dispermic eggs)	Piko, 1958
	Mus musculus	Monospermy; polyspermic eggs extremely rare	Sobotta, 1895a

TABLE 29 (Continued)

Phylum, class, order	Species	Number of spermatozoa penetrating egg	Author
		Monospermy (2 dispermic eggs — 1.2% — out of 178)	Braden et al., 1954
		Monospermy (30 polyspermic, mainly dispermic, eggs — 0.9% — out of 3,377 fertilized eggs)	Braden, 1957
	Cricetus (Mesocricetus) auratus	Monospermy (1 dispermic egg — 1.5% — out of 67)	Ludwig, 1954
		Monospermy (10 dispermic eggs — 1.4% — out of 725)	Austin and Braden, 1956
		Monospermy (4 polyspermic eggs — 3.8% — out of 106 fertilized eggs)	M. C. Chang and Fernandez-Cano, 1958
	Microtus agrestis	Monospermy (1 dispermic egg — 1.7% — out of 59 eggs at pronuclei stage)	Austin, 1957
	Meriones unguiculatus	Monospermy (not one polyspermic egg among 114 at pronuclei stage)	Marston and Chang, 1966
Lagomorpha	Oryctolagus cuniculus	Monospermy (not one polyspermic egg out of ~ 80 eggs)	Yamane, 1937
		Monospermy (1 presumably polyspermic egg — 1.4% — out of 69 at pronuclei stage)	Austin and Braden, 1953
		Monospermy (2 dispermic eggs — 1.1% — out of 189 fertilized eggs)	Dazier and Thibault (see Piko, 1961)
Carnivora	Mustela (Putorius) furo	Monospermy (1 egg — 1.4% — with 3 pronuclei out of 71 eggs at pronuclei stage)	Mainland, 1930
	Felis catus	Monospermy (1 egg — 2.8% — with 3 pronuclei out of 36 eggs at various fertilization stages)	R. Van der Stricht, 1911
Artiodactyla	Sus scrofa	Monospermy (1 egg — 1.6% — with more than two pronuclei out of 64 eggs at pronuclei stage)	Hancock, 1959
		Monospermy (1 dispermic egg — 1.8% — out of 54)	Thibault, 1959

third division stages was noted by Persov (1953b), who considered this phenomenon an acceleration of embryogenesis due to insemination by an excessive amount of sperm.

TABLE 30. Changes in percentage of fertilized eggs with excessive number of blastomeres during initial cleavage divisions (in Acipenser güldenstädti colchicus)

Stage	Number of eggs	Fertilized eggs, %	Eggs with excessive blastomeres, % of fertilized eggs
I division	432	90.3	3.3
II division	499	90.2	7.8
III division	424	89.4	7.7

Eggs forming 4 blastomeres at the second division stage (Figure 54, a') are virtually all monospermic; excess blastomeres at the third division stage in such eggs were very rare. The in vivo observations of formation of cleavage furrows are confirmed by counts of eggs with different numbers of blastomeres at consecutive stages of development. For this, various batches of Acipenser güldenstädti eggs, inseminated with a concentrated spermatozoan suspension, were fixed at the first, second and third division stages, and the percentage of eggs with excessive blastomeres was calculated (Table 30). It was found that the percentage of such eggs increased markedly from the first to the second division and remained unchanged thereafter. On the other hand, the percentages of eggs with excess blastomeres at the second cleavage stage of eggs from the same insemination containing supernumerary spermatozoa or male pronuclei are very close (Ginzburg, 1957a).

Thus, the distinct differences in the cleavage patterns of monospermic and polyspermic eggs of sturgeons make it possible to distinguish between these two groups after the second cleavage stage. At this time, all the polyspermic eggs, depending on the number of penetrating spermatozoa, are distinguished by a larger number of blastomeres than monospermic eggs (compare Figure 54, a' and b'—e').

In this respect sturgeons differ from many other monospermic animals in which polyspermic fertilization partially or completely suppressed cleavage. Retardation of cleavage was noted in the case of pathological polyspermy in ascarids, echinoderms, ascidians, lancelets, frogs, etc. (see below). As a rule, eggs of these animals cannot undergo cleavage in cases of very intensive polyspermy. The lack of suppression of cleavage in sturgeon eggs is due to the fact that polyspermy is never intensive since egg membranes are impermeable to spermatozoa and the number of micropyles is limited.

The presence of excessive numbers of blastomeres at the second and third cleavage stages was traced in polyspermic sturgeon eggs. In two experiments, several hundred polyspermic eggs of Acipenser güldenstädti were selected and incubated under normal fishery conditions (Yushchenko's apparatus). Monospermic eggs having 4 or 8 blastomeres at the respective stages served as controls. During embryonic development

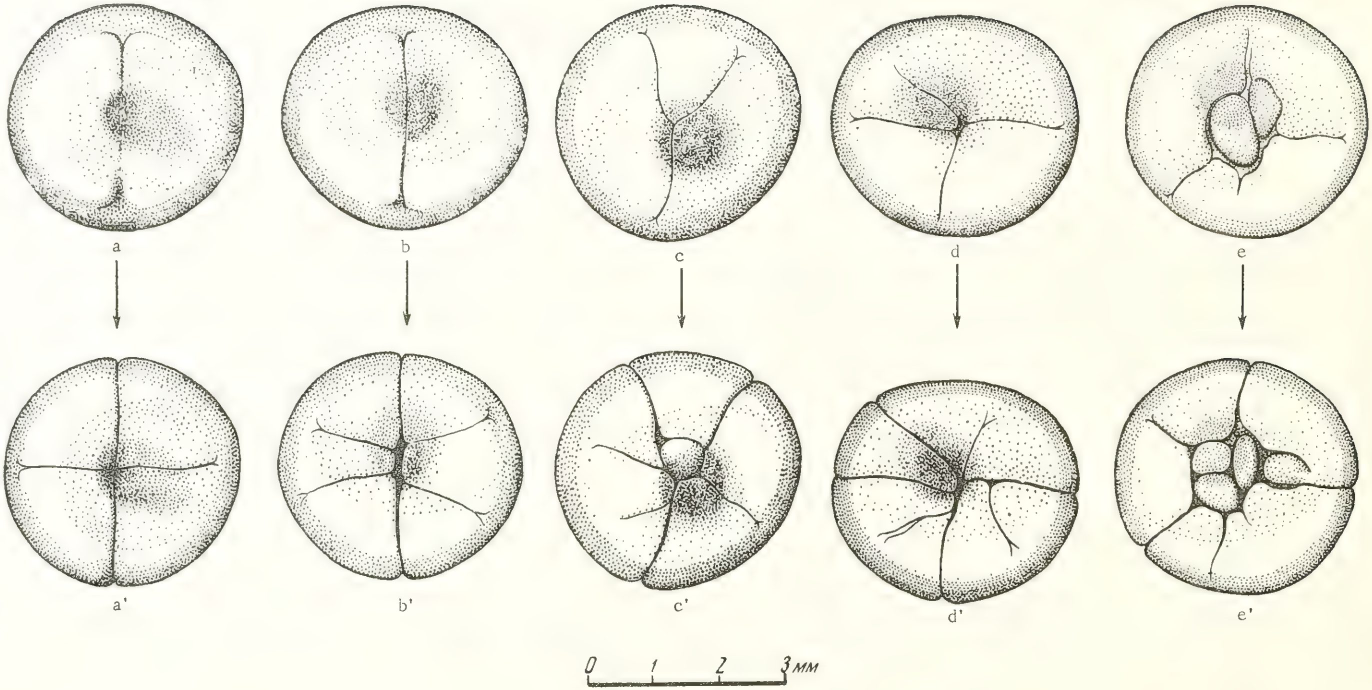

FIGURE 54. Eggs of Acipenser güldenstädti at first (a—e) and second (a'—e') cleavage division stages: a, a' — normal monospermic eggs; the remainder with an excessive number of blastomeres.

82 and 93% of the polyspermic eggs died, while the loss in the control was only 2 and 3% (Ginzburg et al., 1963).

The development of more than 200 polyspermic eggs of Acipenser güldenstädti and A. stellatus was also followed individually under more favorable laboratory conditions (Ginzburg, 1953). The vast majority of embryos developed very atypically. In some cases development was arrested at the gastrula stage; in others gastrulation was delayed and disturbed with occasional partial exogastrulation and formation of a spina bifida. When gastrulation was retarded, the neutral plate was often laid down in the embryo having a large yolk plug; moreover, the plate was shortened and curved. In later stages, there were various disruptions of morphogenesis, such as microcephaly and incomplete separation of the head, shortening and curvature of axial organs, defects in the fin border, dropsy of the pericardium, occasional absence of the heart, etc. About half the embryos died even before hatching began in the control. Among surviving embryos, most were clearly expressed monsters and only a few were capable of hatching independently. Various defects were discovered in the early larval stage in the few polyspermic embryos which seemed normal at hatching; the brain, gills, barbels and mouth developed asymmetrically, the tail was twisted, and a general retardation of growth and delay in yolk resorption were observed.

Although all the polyspermic eggs developed atypically, the degree of the disorders varied according to the intensity of polyspermy. Eggs which completed the first cleavage normally (mainly dispermic) showed fewer deviations and reached later stages than eggs which divided into 4 or 5 blastomeres at the first division.

The harmful effect of polyspermy is also revealed, though less distinctly, in results of incubation of eggs inseminated with variously diluted sperm; the loss percentage rises when the proportion of sperm is increased above a certain level (Persov, 1954a, b).

When sturgeon eggs are inseminated with highly diluted sperm, it was observed cytologically that the egg is invariably penetrated by only one spermatozoon. Insemination with a concentrated suspension in spermatozoa leads to a certain percentage of polyspermic fertilization. The supernumerary spermatozoa participate in development, which leads to profound disturbances of morphogenesis and death of the embryos.

How can the discrepancy between these observations and Persov's conclusion that fertilization in sturgeons is physiologically polyspermic be explained?

Persov discovered spermatozoa in microscopic sections of Acipenser ruthenus eggs which had not changed their structure long after insemination. These lay deep in the cytoplasm, sometimes almost in the center of the egg. In contrast all the spermatozoa in our experiments which penetrated the egg were located only at a very shallow depth in the cytoplasm, where their heads were rapidly and synchronously transformed into vesicular male pronuclei. Only after this transformation was further movement of the sperm complexes deep into the egg observed. This suggests that the unchanged spermatozoa observed by Persov were drawn mechanically into the cytoplasm from the surface, most probably by the flow of fixative in the preparations. This seems quite probable since the cases mentioned belong mainly to series in which Acipenser ruthenus eggs inseminated with highly concentrated sperm were fixed in Carnoy fluid and transferred directly to alcohol. A large quantity of adhering spermatozoa could be preserved on the surface membranes. In our experiments the eggs were fixed mainly in Sanfelice fluid and flushed for two days in running water; thus, even when inseminated with a concentrated spermatozoon suspension, unchanged spermatozoa deep in the cytoplasm were never observed.

This led to the conclusion that fertilization in sturgeons is physiologically monospermic. This conclusion, based on experimental results, is supported by information on the spawning of sturgeons. They spawn in rapid currents where the sperm ejected by the male is instantaneously dispersed by the water. Under such conditions the probability of polyspermic fertilization is negligible. This also explains the preservation of numerous micropyles in sturgeon eggs during evolution. Such a structural feature increases the probability of fertilization in low concentrations of spermatozoa. However, this feature may cause polyspermic fertilization in some eggs when artificially inseminated with concentrated spermatozoan suspensions and thus lead to their elimination.

4.4. TELEOSTS

In the vast majority of teleosts the gametic encounter occurs in water, and only in a few (see p. 66) in the female. Fertilization has been studied only for the first group.

In teleosts with external fertilization, the egg membranes are impermeable to spermatozoa and are furnished with a single micropyle located in animal pole. The diameter of the terminal canal of the micropyle corresponds to the width of the sperm head (see Table 14), so that no more than one spermatozoon at a time can approach the cytoplasm.

Cytological investigations of fertilization, together with in vivo observations of the gametic encounter in several species having small eggs with transparent membranes, indicate that the egg is always penetrated by only one spermatozoon. This was demonstrated in representatives of various orders (see Table 28).

Among all the literature available on this subject, only two cases were interpretated as being due to polyspermic fertilization.

Blanc (1892, 1894) studied fertilization in the lake trout, mainly with whole-mount preparations of exposed blastodiscs (he used microscopic sections very sparingly only for control). These investigations contained many errors, one of which was the conclusion that physiological polyspermy is characteristic for trout based on the following data. In whole-mount preparations of several trout eggs fixed $1\frac{1}{4}$ hours after insemination, a large number of sperm heads scattered randomly over the entire surface of the blastodisc retained their original shape. In eggs fixed 6 hours after insemination, vacuolized sperm heads were sometimes found, while when fixed later no sperm heads were found, which Blanc attributed to resorption. Blanc apparently did not find supernumerary spermatic elements in microscopic sections of blastodiscs; in any event, he gave no evidence that the "supernumerary spermatozoa" he described actually penetrate the cytoplasm. Most likely these spermatozoa were located on the surface of the blastodisc which they reached from the perivitelline space or from the outer membranes. In this material there were undoubtedly many spermatozoa on the membranes, since the eggs inseminated with a concentrated spermatozoan suspension were transferred from the fixative directly to 80° alcohol without washing.

In a second investigation, conducted by Kanoh (1953), eggs of Clupea harengus pallasi were studied. Microscopic sections of several eggs, fixed soon after insemination, showed heads of one or two spermatozoa lying at the cortical surface in addition to the fertilizing spermatozoon embedded in the cytoplasm. In sections of eggs fixed at later stages, Kanoh never observed either degenerating sperm heads or additional male pronuclei, as he assumed that the supernumerary spermatozoa, having penetrated the egg, were soon ejected from the cytoplasm into the perivitelline space. However, such a method of elimination of supernumerary spermatozoa has not been observed in other vertebrates. Considering the position of the heads of supernumerary herring spermatozoa in the few eggs in which they were found, it is more likely that no true penetration of the cytoplasm by the spermatozoa occurred, but that they passed through the micropyle to under the membrane at the moment when the perivitelline space was being formed, and were pressed into the surface of the cortical layer by dessication of the perivitelline space during fixation. This explanation seems quite likely since Pacific herring spermatozoa sometimes penetrate through the micropyle of the fertilized egg and remain suspended in the perivitelline fluid.

In any case, not a single reliable case of polyspermic fertilization was established in intact eggs of various teleost species in which the heads of supernumerary spermatozoa embedded in the cytoplasm were either transformed into male pronuclei or degenerated. Experiments to achieve polyspermy in teleosts by insemination of eggs with concentrated sperm, as in sturgeons, produced no results. In experiments on Oryzias latipes (Sakai, 1961) and lake trout (Ginzburg, unpublished) fertilization was invariably monospermic even when highest possible concentrations of active spermatozoa were used.

Multiple penetration of teleost spermatozoa was observed only in experiments with naked eggs after mechanical or proteolytic removal of the egg membranes. Such experiments were performed on Pacific herring (Yanagimachi, 1957d; T.S. Yamamoto, 1958), chum salmon (Kanoh and Yamamoto, 1957), lake trout (Ginzburg, 1960b, 1961a), Oryzias latipes (Sakai, 1961) and goldfish (Chu-Su-hwai et al., 1964). The supernumerary spermatozoa were invariably involved in development, which led to disruption of cleavage with formation of distorted blastomeres and, subsequently (Yanagimachi, 1957d), to delayed gastrulation and early death of the embryo, as is characteristic of pathological polyspermy.

Experiments with intact and naked eggs showed clearly that fertilization in the teleosts studied is monospermic.

No data have been published on the gametic association in fishes with internal fertilization. A cytological investigation begun on fertilization in live-bearing platys, Poecilia reticulata and Xiphophorus helleri (Ginzburg, unpublished data), encountered great difficulties because fertilization of eggs in different females by artificial insemination took from a few days to 6—7 days after the sperm entered the vagina. With such variability, only by chance was it possible to obtain eggs at the early stages of development. Out of more than 400 females, only 4 (guppy) had eggs at the stages of approach and fusion of the pronuclei, and in 14 (7 guppy and 7 swordtail) at the early cleavage stages. No supernumerary spermatozoa (neither unchanged sperm heads nor male pronuclei) were found in any of these cases.

Among fishes fertilization is physiologically polyspermic in selachians and chimaeras, physiologically monospermic in sturgeons and teleosts. To clarify the general patterns of monospermic and polyspermic fertilization, data on numbers of spermatozoa penetrating the egg in other groups of animals will now be examined.

4.5. INVERTEBRATES

4.5.1. Sponges

The internal fertilization in sponges is effected by an extremely unusual indirect method (Gatenby, 1920; Duboscq and Tuzet, 1932, 1937, 1942, 1944; Tuzet, 1947, 1950b) in which spermatozoa are conveyed by the water system of canals perforating the sponge body from one individual to another. The young oocytes located in the parenchyma were separated from the flagellate chambers, lined with choanocytes. The spermatozoa actively attach to the plasmic collar of the choanocyte and enter the cytoplasm where they are transformed. The tail is lysed, the head and middle piece swell and surround the membrane, and appear to be encysted, which led Duboscq and Tuzet to call it the "spermiocyst". The choanocyte containing the spermatozoon loses its collar and flagellum, forming an amoebocyte, and migrates into the parenchyma. There the cell bearing the "spermiocyst" approaches the oocyte (which is at the stage of slow growth or, in other species, the beginning of the rapid growth) and combines with it. The spermiocyst remains in the ooplasm but does not change until the beginning of the second maturation division when the sperm head sheds the membranes, is transformed into a male pronucleus and fuses with the female pronucleus.

Fertilization was regularly monospermic in all the calcareous and siliceous sponges studied. Rare cases of dispermy were found in two species of calcareous sponges. Isolated eggs each had 3 pronuclei in Sycon raphanus, whereas no such eggs were found in Grantia compressa but an oocyte was found with two spermiocyst cells simultaneously attached; thus, there is the possibility of formation of supernumerary pronuclei as a result of polyspermic fertilization (Duboscq and Tuzet, 1937). The fate of dispermic eggs is unknown.

4.5.2. Cnidaria

Many free-living jellyfishes eject sexual products into the water where the gametic association takes place. Fertilization occurs in the parent organism in some Scyphomedusae, in hydroids with attached medusoids and in cnidarians lacking the medusoid generation (Hydrozoa, Actinoza).

As a rule, an egg is penetrated by a single spermatozoon (Tables 28 and 29), as for example in Sympodium coralloides. Spermatozoa, carried by the water into the oral cavity, penetrate through the endodermal wall and approach the oocyte. Each oocyte is surrounded by a mucous capsule attached to the pedicle. The spermatozoa pass through the jelly material of the pedicle and accumulate in a small chamber, remaining in the capsule after resorption of nutritive cells. Although 2–4 spermatozoa generally reach this chamber, the egg is penetrated by only one (Tuzet, 1941).

In some species (Tubularia mesembryanthemum, Pennaria tiarella, Obelia loveni) polyspermy is observed quite often. The fate of polyspermic eggs was not traced in two species mentioned. Fertilization and egg development were studied in detail for tubularians (Benoit, 1925), and led to the conclusion that normal eggs with a single haploid nucleus are each penetrated by only one spermatozoon, whereas fertilization is regularly polyspermic in multinuclear eggs. In the colonies investigated by Benoit, multinuclear eggs occurred at the same frequency as normal ones, and their embryos probably develop normally (at least up to the free-swimming larval stage). Therefore, their fertilization was not regarded by Benoit as pathological, although it has many features similar to pathological polyspermy in other animals. The development of these eggs will be discussed here, since it is one of the very few examples of multiple penetration of spermatozoa, accompanied by multiple karyogamy, which does not lead to disruption of development and death of the embryo.

Oocytes of tubularians grow by means of fusion of a large number of egg cells. Normally, nuclei of all but one of these egg cells degenerate rapidly. However, development sometimes is different and giant multinuclear oocytes appear. At the end of the growth period, such oocytes are divided by furrows into 2 or 3 secondary eggs (a specific case of polyembryony). Besides the principal female nucleus, there are about ten oocytic nuclei in such eggs, which have not degenerated. All the nuclei in the secondary eggs end maturation division synchronously, after which several (2–6) female pronuclei form groups like polykaryons. As a result, there is always a normal haploid female pronucleus and two or three polyploidal nuclei in the secondary eggs. Mature eggs enter the cavity of the gonophore and encounter the spermatozoa produced by the male colony. Secondary eggs are always penetrated by several (often about 15) spermatozoa, whose heads are transformed into male pronuclei, some of which, being closest to the animal pole, fuse with the normal haploid female pronucleus. The remaining male pronuclei, located mainly in the vegetal pole, fuse into polyploidal nuclei around which develop several seminal asters (corresponding to the number of fusing nuclei). These patterns are very similar to the fusion of male pronuclei sperm often observed in highly intense pathological polyspermy in the frog (Brachet, 1910a, b). However, the course of development of the polyspermic egg in tubularians is very peculiar. The female and male polykaryons fuse. Then the polyploidal synkaryons divide repeatedly through multipolar mitoses, whose patterns are gradually normalized as development proceeds. According to Benoit's data, the descendants of the polyploidal synkaryons participate in formation of the embryonic body as much as the descendants of the normal diploid synkaryon which divides by bipolar mitoses.

4.5.3. Flatworms

Fertilization is internal in this group of worms. The fertilization process has been studied fairly thoroughly for several turbellarians and many digenetic trematodes (Table 29). The egg is regularly penetrated by only one spermatozoon. In the turbellarians Eustylochus ellipticus and Planocera nebulosa (Van Name, 1899) and in the trematode Zoogonus mirus (Goldschmidt, 1905; Wasserman, 1913) isolated eggs containing several spermatozoa were found as a rare exception. The subsequent fate of such eggs was not followed. Two male pronuclei were sometimes found in the zygote of Fasciola hepatica, but this was observed only under atypical conditions when no shell is formed around the oocyte (Bogomolova, 1956).

Available data tend to confirm that physiological monospermy is characteristic for most flatworms. Among forms examined, the turbellarian Dugesia benazzii is perhaps an exception; in one biotype of this species, which reproduces by means of pseudogamy, multiple penetration of spermatozoa was often observed with subsequent emergence of the spermatozoa from the egg or else resorption (Benazzi Lentati, 1953); another exception is Diphyllobothrium latum whose zygotes often contain 3 or 4 pronuclei (Pavlova, 1955).

4.5.4. Roundworms

Fertilization is internal in roundworms, and the gametic encounter occurs in the upper section of the uterus.

The fertilization process was best studied for Parascaris equorum. The unfertilized egg is covered with a thin, but dense vitelline membrane. Van Beneden described a micropyle in this membrane as an aperture filled with granular cytoplasm which he called the "penetration plug." He believed that the ascarid spermatozoon can penetrate the egg only at this point (Van Beneden, 1883). However, these data were subsequently disproved by other investigators who were unable to locate a micropyle in the ascarid egg membrane and concluded that the structure described by Van Beneden was probably an artifact of poor fixation or crushing of the egg. It was demonstrated that the spermatozoon can penetrate the egg surface anywhere, apparently, by dissolving the egg membrane at the point of contact (Zacharias, 1887; Boveri, 1888).

Fertilization in Parascaris equorum is monospermic in the vast majority of cases, whereas penetration by several spermatozoa is rare (see Table 29). Among many thousands of eggs examined, only 6 dispermic eggs were found by Van Beneden and 2 by Boveri, while no eggs were found with a greater number of spermatozoa. Zacharias occasionally found eggs containing 2–3 or more spermatozoa (up to a maximum of 10), which were distinguished by the atypical structure of membrane and nucleus, and were weakened or abortive as Zacharias surmised. Makarov found in Parascaris equorum no more than 1% dispermic eggs (1957b) and very rarely eggs with a greater number of spermatozoa of up to 8 (1957a, b, 1964).

All the spermatozoa penetrating the egg form similar vesicular nuclei with seminal asters. Multipolar mitoses occur in the polyspermic eggs at the first cleavage division; in dispermic eggs there are 4 cytocentra, and up to 12 cytocentra are encountered when several spermatozoa penetrate (Makarov, 1957a). The number of cytocentra is not always twice the number of penetrating spermatozoa, and it is observed that division of the seminal aster in two does not occur in every case (Sala, 1895). Polyspermic eggs divide directly into several (usually 3–4) blastomeres.

The development of eggs forming 4 blastomeres at the first division (followed by Boveri and Stevens, 1904) proceeds in a distinctly atypical manner. Some of the blastomeres cease dividing early, the embryo assumes an irregular shape and development is suspended (usually not later than the blastula stage, and very rarely after laying down of the archenteron). All the embryos soon die.

Parascaris equorum it is possible to obtain a considerable percentage of polyspermic eggs experimentally by cooling the worms to temperatures from +1 to -6°C. Eggs with 3–4 spermatozoa retain their capacity for development, while eggs penetrated by up to 8–12 spermatozoa generally do not develop beyond the stage of separation of the first polar body and display degenerative changes while still in the uterus (Sala, 1895).

Fertilization is regularly monospermic in another parasitic nematode, Toxacara canis (Kul'chitskii, 1888), as in Parascaris equorum; only in few cases is the egg penetrated by two spermatozoa.

In free-living nematodes of the genus Rhabditis — R. aberrans (Krüger, 1913) and R. monohystera (Belar, 1924) — monospermy was also proven.

The rarity of polyspermic eggs in normal fertilization conditions and their sharply atypical development indicate that roundworms are physiologically monospermic.

4.5.5. Segmented Worms

In annelids of various classes, the egg is regularly penetrated by only one spermatozoon; this applies both to species with external fertilization, which constitute the great majority, and to those few species with internal fertilization (see Tables 28, 29). Oligochaetes are perhaps an exception, and shall be dealt with more thoroughly later.

Monospermy is characteristic for the whole class of polychaetes. In Nereis limbata which was studied in detail by Lillie (1911, 1912, 1913a), hundreds of spermatozoa attach to the egg membrane of inseminated eggs, but all but one are washed to the egg surface within a few minutes by the flow of discharging mucus; polyspermy is observed extremely rarely.

In the hermaphroditic polychaete Ophryotrocha puerilis, cross-fertilization is normal. This is ensured by the different maturation times of eggs and sperm. Sexual products are discharged into the water where gametic association takes place. The egg is normally penetrated by only one spermatozoon (Korschelt, 1895). Rarely masses are encountered (containing weakened eggs according to Korschelt) in which a considerable percentage of polyspermy is observed. All the spermatozoa change synchronously in polyspermic eggs and form male nuclei which fuse with the female pronucleus. As described above for polyspermic sturgeon eggs, multipolar mitotic figures with irregularly arranged chromosomes are formed when the egg is penetrated by groups of 3—6 nuclear elements. Cleavage of polyspermic eggs always is atypical, the blastomeres differ greatly in size and some of the eggs often remain undivided. The nuclei in multinuclear blastomeres are juxtaposed and may fuse. Some polyspermic eggs are completely incapable of cleavage.

In Ophryotrocha the egg and spermatozoon sometimes mature simultaneously in a single segment of the body; self-fertilization occurs in some individuals and the eggs begin cleavage in the body cavity of the female. Development of such eggs is accompanied by disruptions characteristic for polyspermy.

Fertilization is normally monospermic in Pectinaria gouldii. Sometimes penetration is observed of two or, extremely rarely, 3—4 spermatozoa (Austin, 1963). In such polyspermic eggs multipolar mitoses occur at the first cleavage division, as in Ophryotrocha.

We shall dwell briefly on polychaetes characterized by internal fertilization. In Myzostomum parasiticum, a symbiont of sea lilies, insemination is by dermal impregnation and the gametic encounter occurs in the female genital tract (Prenant, 1959). The egg is generally penetrated by one spermatozoon and only very rarely (3 eggs in many thousands) by 2 spermatozoa (Kostanecki, 1898). Two male pronuclei are formed in dispermic eggs. The subsequent fate of such eggs has not been traced.

In the two species of Archiannelida studied, Saccocirrus papillocercus (Buchner, 1914) and Dinophilus apatris (Nachtsheim, 1919), the spermatozoon penetrates the egg cell at an unusually early stage, long before maturation of the oocyte is completed; fertilization is monospermic.

These data contradict the observations of I.I. Kiknadze (1956) that the polychaete Phyllodoce maculata often shows polyspermic fertilization. Kiknadze did not observe formation of additional male pronuclei; the fate of polyspermic eggs of Phyllodoce is unknown.

In the few members of the classes Hirudinea and Echiurida in which fertilization has been investigated, the egg is generally penetrated by only one spermatozoon, as in polychaetes.

Fertilization in oligochaetes is distinguished by specific features. The egg was usually penetrated by one spermatozoon in the two representatives studied, but sometimes more spermatozoa were observed: in Rhynchelmis limosella up to 6 (Vejdovský, 1888—1892), in Eisenia (Allolobophora) foetida up to 3 and, rarely, up to 9 spermatozoa (Foot, 1894, 1898; Devriès, 1964a).

Fertilization occurs outside the genital tract of the female in Eisenia, as in other lumbricids, but not simply in the outer medium. The eggs are laid in a cocoon formed from a secretion of the clittelium, to which is added sperm of another worm, obtained at a previous copulation of two hermaphroditic individuals, gametic association occurs from the spermatheca; thus, within the secretion of the glands of the female.

Polyspermy of various intensity can easily be obtained on artificial insemination of Eisenia eggs. Study of the cytology of development of polyspermic eggs by Devriès (1964a, b) led to unexpected results. When the number of penetrating spermatozoa does not exceed 6 (i. e., an intensity of polyspermy of the same order observed under natural conditions), the initial transformations of the spermatozoa proceed exactly as in pathological polyspermy in many animals. The sperm heads change synchronously into male pronuclei which all fuse with the female pronucleus. In prophase of the first cleavage division, the number of seminal asters in the cytoplasm is twice that of penetrating spermatozoa.

In metaphase both isolated bipolar and also multipolar mitotic figures are formed. However, the changes in anaphase of the first division are unusual for such a process; the principal spindle, with a diploid set of chromosomes develops progressively and divides, while supernumerary spindles, asters and chromosomes are rapidly resorbed. Subsequent development of the eggs proceeds quite normally as in monospermic fertilization.

On the otherhand, if a large number (6–15) of spermatozoa penetrate the Eisenia egg, a main spindle does not appear, chromosomes are arranged randomly and the eggs die without cleavage. In still more intensive polyspermy, transformation of the spermatozoa ceases early or is blocked completely.

The low intensity of polyspermy and the rare occurrence of polyspermy following natural fertilization, as well as the mechanism which prevents supernumerary spermatozoa from developing, led to the assumption that the type of fertilization in Eisenia is facultative physiological polyspermy, which is also inherent in many insects.

4.5.6. Arthropods

Among arthropods, detailed investigations into the fertilization process have been conducted only for insects.

Insect eggs are covered with a delicate vitelline membrane and a dense chorion which varies in thickness and hardness, but is always impermeable to spermatozoa. The spermatozoa penetrate via micropylar apertures in the chorion or through thin sections of the chorion. In most cases the micropyles are located at the anterior end of the elongate egg (Weber, 1949), and their number varies considerably. There is only one micropyle in Drosophila melanogaster (Nonidez, 1920) and other species of this genus (Counce, 1959a); in Pyrrocoris apterus there are 4–9 (Henking, 1892), 12–14 in Kalotermes flavicollis (Truckenbrodt, 1964) and about 30 in Melanoplus differentialis (Slifer, 1958).

Fertilization is internal in insects. On mating the sperm enters the vagina in the form of spermatophores or free spermatozoa distributed in the seminal fluid. The spermatozoa usually penetrate the micropylar canals at the moment when the egg, advancing along the genital ducts of the female, passes the efferent duct of the spermatheca (Weber, 1949).

In early investigations of fertilization in insects it was shown that the egg is often penetrated by several spermatozoa. This was first noted in the bluebottle and honeybee by Blochmann (1887, 1889) and was thoroughly investigated by Henking (1892) who established polyspermy in 6 species belonging to Heteroptera, Lepidoptera, Coleoptera and Hymenoptera (see Table 29). In his work, which has remained one of the best on this subject, Henking wrote that the egg in Pyrrhocoris apterus, Agelastica alni and Lasius niger is regularly penetrated by several spermatozoa through the micropylar canals; he counted 6–7 or more spermatozoa per egg in the outer layer of cytoplasm (periplasm) of fresh eggs of Pyrrhocoris and Agelastica. In later stages Henking found a smaller number of male nuclear elements (not more than 3) in cytoplasmic islands among the yolk of all the species studied. He considered it likely that only some of the penetrating spermatozoa become embedded in the yolk and are transformed into male pronuclei, while most remain in the surface layer and degenerate there.

The existence of polyspermy in the bee was confirmed by Petrunkevich (1901), who often found 2–3 spermatozoa, sometimes even up to 7, in fertilized eggs from which workers developed.

Since polyspermy was discovered in almost all species studied in the first works on fertilization in insects, the concept developed that it is a constant and widespread phenomenon (see, for example, Depdolla, 1928; Johannsen and Butt, 1941; Weber, 1949). Later, however, it was established that there are considerable differences in this feature between different insect species (see Table 29). For example, penetration by several spermatozoa is the rule in Bombyx mori and Parum colligata (85 and 75% polyspermy), 65% of the eggs are polyspermic in Cochliomiya hominivorax, while there are only about 10% in Kalotormes flavicollis, about 3% in Pseudococcus citric, 1% in Habrobracon juglandis; in many species (Diacrisia virginica, Copidosoma gelechiae, Paracopidomopsis, Platygaster dryomiae, Drosophila virilis, Sciara impatiens, Dacus tryoni) no cases of polyspermic fertilization were observed. It may therefore be assumed that polyspermy in these species is only a rare exception.

Contradictory data were obtained for Drosophila melanogaster. According to earlier data (Huettner, 1924, 1927; Sonnenblick, 1950; Counce, 1959b), almost all eggs of this species are polyspermic and from 5 to 12 spermatozoa or more are present in the egg. However, Hildreth and Lucchesi (1963) found only about 5% dispermic eggs in D. melanogaster and not a single case of more than 2 spermatozoa. They explained these discrepancies by the inadequate investigation methods of their predecessors; on microscopic sections stained with ferric hematoxylin, parts of the very long, strongly twisted sperm tail (1.75 mm long in D. melanogaster) could be mistaken for supernumerary spermatozoa. Hildreth and Lucchesi used Feulgen reaction and also inseminated eggs with spermatozoa marked with ^{3}H-thymidine, which prevented such an error. Counce (1959b), who usually found 5–6 spermatozoa in eggs of 5 species of Drosophila, including D. melanogaster, 3–4 times more in D. funebris and D. hydei and 50–100 spermatozoa in those of D. americana and D. virillis, reinvestigated her own material after Hildreth and Lucchesi indicated the error was hers. Counce then concluded that eggs of all species of Drosophila contain not more than 2 spermatozoa (see Hildreth and Lucchesi, 1963).

The frequency of polyspermic fertilization in different insect species varies greatly, while variations in the intensity of polyspermy are less significant. In most cases the egg is penetrated by no more than 2–3 spermatozoa, and much less often up to 8–11.

Henking (1892) already demonstrated that penetration by several spermatozoa has no harmful effect on the development of the insect egg. This is indicated that the number of eggs incapable of normal development among polyspermic eggs. In Pyrrhocoris about 50% of eggs contain 2–3 spermatozoa each, whereas only 2–4% of eggs die before larvae hatch. There is no difference among larvae which have developed from monospermic and polyspermic eggs.

However, pathological changes occur if the intensity of polyspermy is greater than usual. Huettner (1927) counted hundreds of spermatozoa per egg, while the filiform sperm heads were still unchanged in Drosophila melanogaster where a very large number of spermatozoa penetrate eggs. It is possible that an error connected with the difficulty in determining the number of unchanged spermatozoa on microscopic sections of Drosophila stained with hematoxylin is involved, and that the true number of penetrating spermatozoa was small. However, whether the number was several hundreds or thousands of spermatozoa, it is evident that excessive polyspermy is accompanied by multipolar mitoses and disorganization of the maturation divisions, and may cause profound disruptions in the further development of the eggs.

We shall now deal in somewhat more detail with the fate of supernumerary spermatozoa in the case of the polyspermic intensity normal for insects. According to Henking's data mentioned above, only some of the penetrating spermatozoa in Pyrrhocoris, Agelastica and Lasius reach deep in the egg and form male pronuclei, while the remainder degenerate in the outer layer of cytoplasm. In polyspermy in Phragmotobia fuliginosa, Lymantria dispar, L. japonica and L. monacha sometimes only one of 2–3 spermatozoa penetrating the egg enters deeply and forms a male pronucleus, while the remainder lyse. Usually, however, all these spermatozoa enter simultaneously and undergo the same transformations, so that until one of the male pronuclei approaches the female pronucleus it is impossible to determine which participates in karyogamy (Seiler, 1914). In Apis mellifera (Nachtsheim, 1913), Bombyx mori (Bataillon and Tchou-Su, 1928, 1930, 1933; Tchou-Su, 1953), Parum colligata (Tchou-Su and Chang-Ko, 1951) and Cochliomyia hominovorax (Riemann, 1965), all the spermatozoa which penetrated the egg changed synchronously and formed vesicular male pronuclei accompanied by a seminal aster.

The fate of the spermatic complexes was the same in all the species studied. One male pronucleus, nearest to the female pronucleus, fuses with it, the seminal aster divides and cleavage division proceeds in the synkaryon. The supernumerary spermatic complexes also begin to divide, but the mitotic figures are blocked at metaphase and rapidly degenerate.

The processes accompanying cessation of development of the supernumerary male pronuclei were studied in detail for Bombyx mori and Parum. They are distinguished by a very peculiar feature; the cytocentra separate from the blocked karyokinetic figure which is in a state of pycnosis. For a time the cytocentra divide independently and give the impression of nuclear cytoplasmic islands. Later, all the derivatives of the supernumerary spermatozoa disappear.

In some cases the mechanism preventing development of supernumerary spermatozoa is disrupted, and gynandromorphic individuals emerge with androgenetic male parts.

Gynandromorphs are known in many insect species, but appear very rarely. The original of gynandromorphs has been debated for centuries, and very different explanations have been given. The discovery of a special line of honeybees having a considerable percentage of spontaneous gynandromorphs, made it possible to investigate experimentally a case of this disruption (Rothenbuler et al., 1952). It was possible to show by genetic analysis that the female parts of gynandromorphic bees are of zygotic origin, and the male parts are androgenetic and develop from the supernumerary spermatozoa. This is apparently the origin of several mosaics of Habrobracon juglandis obtained in a particular line also regularly producing gynandromorphs (Whiting, 1943). It may be assumed that in mutant strains of bees and Habrobracon the egg lost the capacity for producing the factor normally inhibiting mitotic activity of supernumerary spermatozoa.

The investigations established the following features of polyspermy in insects: 1) polyspermy varies, so that a larger or smaller percent of the eggs are monospermic, which predominates in many species; 2) the intensity of polyspermy is low; 3) as a rule, polyspermic fertilization does not disrupt development. For this type of fertilization Henking proposed the term "facultative polyspermy." Facultative polyspermy is also inherent in other animals, e. g., gastropods, which indicates the diversity of physiological polyspermy.

Very little is known concerning fertilization in other groups of arthropods.

In the class Crustacea both external and internal fertilization occurs. In Amphipoda the gametic encounter occurs outside of the genital ducts of the female. Investigations performed on Gammarus duebeni and Orchestia gammarella showed that the egg is penetrated by only one spermatozoon (Le Roux, 1933; Campbell-Parmentier, 1963).

In contrast to Amphipoda, the oocytes in Artemia salina, after completing vitellogenesis, are fertilized in the ovary. The egg is usually penetrated by only one spermatozoon, but polyspermic eggs have also been encountered (Fautrez-Firlefyn, 1951).

Fertilization is also internal in Arachnida. Eggs of Theridion tepidariorum are quite frequently polyspermic (Montgomery, 1907). At stages of formation and fusion of pronuclei, 1 to 4 male pronuclei, identical in structure and size, are found in the egg. One pronucleus fuses with the female pronucleus. During the first cleavage division the supernumerary sperm nuclei form haploid mitotic figures. Their subsequent fate has not been traced.

It is very likely that fertilization in Artemia and Theridion, as in insects, is facultative polyspermic.

4.5.7. Mollusks

Fertilization has been studied most thoroughly for the class Gastropoda, particularly for the subclass Pulmonata, which are hermaphroditic like most gastropods. The gametic encounter takes place in the dilated section of the duct of the hermaphroditic gland, in the fertilization chamber or in the gonad itself before ovulation of the oocytes (Lams, 1910; Crabb, 1927; Perrot, 1939).

There is generally only one spermatozoon or one male pronucleus in eggs of Pulmonata fixed at the moment of laying or later (Kostanecki and Wierzejski, 1896; Wierzejski, 1905; Larambergue, 1930; 1939; Perrot, 1939). However, several spermatozoa per egg were found in the ooplasm at earlier stages.

The presence of several spermatozoa in the egg, only one of which forms a male pronucleus, was already described for Arion empiricorum by Platner (1886) and for Helix aspersa by Garnaut (1888–1889). The frequency of polyspermy and its intensity vary in different species (see Table 29); 2 spermatozoa are occasionally found in the egg of Lacuna vincta and up to 3 in Helix aspersa; polyspermy is fairly frequent in Helix pomatia and the egg is penetrated by up to 5 spermatozoa; polyspermic fertilization is the rule in Lymnea stagnalis. In all these mollusks the supernumerary spermatozoa in the cytoplasm disintegrate rapidly. In Lymnea stagnalis the heads of supernumerary spermatozoa swell and are dissolved when the fertilized oocyte moves along the efferent duct of the hermaphroditic gland, so that only one spermatozoon is seen in the egg after it is laid (Crabb, 1927; Bretschneider, 1946, 1948).

Cases have been described where supernumerary spermatozoa are not resorbed, but form additional male pronuclei. Such eggs develop abnormally and die. These cases seem to contradict the data presented above and indicate that polyspermy is pathological in gastropods. However, formation of several male pronuclei was observed only rarely and under abnormal conditions. In Limax agrestis, several male pronuclei (up to 12) were found in each "polyvitelline egg" in which a large number of abortive oocytes are covered by a common capsule; such polyspermic eggs usually do not begin cleavage and show signs of degeneration (Byrnes, 1899). Multipolar mitoses, characteristic for pathological polyspermy, were encountered in Physa fontinalis in undivided and atypically cleaved eggs of normal capsules (containing one egg); such disruptions were usually found in eggs of starved or parasite-infested snails, or in snails intentionally kept in deliterious conditions (Wierzejski, 1905).

On the basis of available data it seems likely that gastropods have facultative polyspermy like insects. Polyspermic eggs were encountered in different species in varying proportions. Polyspermy distinguished by low intensity generally does not involve disruption of development. This is achieved by the elimination of supernumerary spermatozoa in the earliest stages of development, even before the heads are transformed into male pronuclei.

In most bivalve mollusks, the sexual products are discharged into the water. In many freshwater forms (for example, Unio) the female lays eggs in the gills (between the outer and inner layer of the gill) where they are fertilized by spermatozoa carried in by the water current. In all the cases studied (see Table 28) bivalve fertilization is monospermic.

4.5.8. Bryozoa

According to the fragmentary observations of Marcus (1934), the gametic encounter in the freshwater bryozoan Lophopus cristallinus (order

Phylactolaemata) occurs in the ovary at the first maturation division stage. Fertilization was monospermic in all the cases studied.

In the marine bryozoan Membranipora pilosa (order Eurystomata), the oocyte at the metaphase of the first maturation division enters the body cavity where fertilization takes place (Bonnevie, 1907). Bonnevie described the penetration of the oocyte by a large number of spermatozoa (apparently several dozen). The filiform spermatozoa are arranged at first in clusters in the ooplasm, but then separate and are distributed throughout the egg. Bonnevie did not succeed in following the spermatozoa after the egg was laid and maturation divisions were completed. However, since all the fertilized eggs contained many spermatozoa, she concluded that polyspermy in M. pilosa is physiological.

A reinvestigation of this question carried out by Just (1934) showed the error of this conclusion. It appeared that specimens of M. pilosa are easily damaged, and that it is very difficult to obtain eggs in good condition (capable of continuing development). Cytological examination of normally developing eggs showed no signs of polyspermy. Only eggs from injured and dead animals corresponded to Bonnevie's description; such eggs never completed meiosis and were not cleaved. Thus multiple spermatozoan penetration of the M. pilosa egg is a pathological phenomenon.

Owing to the scant data at our disposal, it was not possible to determine definitively the type of fertilization in bryozoans, but it is most probably physiologically monospermic.

4.5.9. Echinoderms

Fertilization is external in echinoderms and the gametic association occurs in seawater. The fertilization process was studied in detail for starfishes and, in particular, for sea urchins.

It was already shown at the end of the last century in the fundamental investigations on fertilization in animals that, the echinoderm egg is normally penetrated by only one spermatozoon (see Table 28). Polyspermy is observed under certain conditions, but is always pathological.

Fol (1877c, d, 1877—1879) in his own investigations on Asterias glacialis showed that fertilization is often polyspermic in cases of insemination of insufficiently mature eggs (with the germinal vesicle still undissolved), overmatured eggs (kept for a long period in seawater) and poor-quality eggs (obtained from starfishes kept in unfavorable conditions). Subsequently, polyspermic fertilization was obtained in sea urchin eggs subjected to the effects of narcotics and other chemical agents and in heated egg (Hertwig and Hertwig, 1887), as well as unharmed, good-quality eggs inseminated with an extremely concentrated spermatozoan suspension (Boveri, 1908).

In cases of polyspermic fertilization the heads of all the spermatozoa which penetrate the egg are transformed synchronously into male pronuclei and are then involved in development.

In dispermic and trispermic sea urching eggs (Hertwig and Hertwig, 1887; Boveri, 1908), all the male pronuclei usually fuse with the female pronucleus;

moreover, tripolar or more often quadripolar mitoses appear and the egg divides directly into 3—4 blastomeres at the first division. Sometimes one of the male pronuclei in a dispermic egg does not participate in fusion but forms an independent spindle (Boveri's "Doppelspindeltypus"). If the spermatic amphiaster is oriented parallel to the spindle of the synkaryon, the first division may appear to proceed quite normally; the egg divides into two blastomeres, each containing two nuclei, which becomes noticeable during subsequent divisions.

In cases of intensive polyspermy (Fol, 1877—1879), only 2—3 male pronuclei fuse with the female pronucleus, while the remaining nuclei form a spindle independently of the synkaryon. Such eggs cleave irregularly and sometimes are quite incapable of cleavage.

Cleaving polyspermic eggs are characterized by a larger number of blastomeres than the monospermic eggs at the same stage. Their subsequent development proceeds atypically. Most of the sea urchin eggs which immediately divide into 4 blastomeres die even before gastrulation begins (Driesch, 1892). Embryos which reach later stages of development usually have sharply pronounced organizational defects. Even dispermic eggs do not develop beyond the pluteus stage (Boveri, 1908).

The data given and numerous later investigations show clearly that fertilization in echinoderms is physiologically monospermic.

4.5.10. Chaetognatha

Representatives of the Chaetognatha are hermaphrodite. Fertilization is internal, assisted by two auxiliary cells or synergids. These cells form stalks which attach the oocytes to the wall of the sperm duct which is filled with spermatozoa from another individual. The stalk is perforated by spirally twisting tubules through which the spermatozoon penetrates until it either reaches the cytoplasm of the oocyte directly, as in Sagitta bipunctata and S. serratodentata (Stevens, 1903; Elpat'evskii, 1913) or enters the cytoplasm of synergid which has fused with the oocyte and then passes into the ooplasm, as described in Spadella cephaloptera (Vasiljev, 1925).

The spermatozoon penetrates the ooplasm at the stage when the first maturation spindle is formed. For a time the spermatozoon undergoes no changes, at the beginning of the second maturation division the sperm head swells and is transformed into a pronucleus. Karyogamy takes place after fertilized eggs are deposited in the water.

Immediately after spermatozoan penetration, the synergid is torn out of the oocyte, and the egg membrane seals the opening left by the stalk attachment.

In all the species of Chaetognatha studied fertilization is monospermic. In Sagitta the tubule piercing the bodies of the auxiliary cells may be ascended by several spermatozoa, but the cytoplasm of the mature oocyte is penetrated by only one of them. By contrast, in immature oocytes many spermatozoa were observed in some cases (Elpat'evskii, 1910, 1913).

4.6. CHORDATES (EXCEPT FOR FISHES)

4.6.1. Enteropneusta

Sexual products are discharged into the seawater where the gametic association takes place. In Saccoglossus kowalevskii, the spermatozoon can penetrate at any point on the egg surface. Fertilization is normally monospermic. Sperm penetration is accompanied by the formation of a well outlined sensory tubercle. Several sensory tubercles are often found in eggs inseminated after a long stay in the water. This is undoubtedly due to polyspermic fertilization. Observations of egg development show that the frequency of polyspermy increases with overripening of the eggs, and the percentage of normal larvae decreases sharply (Colwin, and Colwin, 1953, 1954b).

4.6.2. Tunicata

Fertilization is external in ascidians, as in the enteropneusts. The egg is penetrated generally by only one spermatozoon (Table 28). Fertilization has been most thoroughly studied for Styela partita (Conklin, 1905) in which isolated dispermic eggs are occasionally found. In such eggs both spermatozoan heads form pronuclei synchronously, and one fuses with the female pronucleus. The supernumerary male pronucleus begins mitotic division at the same time as the synkaryon, and produces a bipolar spindle which always remains isolated (multipolar mitoses were not observed). The haploid nuclei may divide a second time, but these divisions are not accompanied by cleavage, and dispermic eggs die without cleaving.

4.6.3. Acrania

Branchiostoma (Amphioxus) lanceolatus discharges gametes into the water — female usually later than male — and their association takes place directly in the seawater. The egg is covered with a thin, delicate membrane permeable to spermatozoa.

Fertilization is generally monospermic (see Table 28). Under certain conditions polyspermy is occasionally observed. If the eggs are kept in water for a certain time before insemination each quite often is penetrated by several spermatozoa (Sobotta, 1897). The same occurs when ovulated eggs remain inside the female, so that fertilization takes place in the atriopore (Van der Stricht, 1895).

Polyspermic eggs are incapable of normal development. At first all the spermatozoa penetrating the cytoplasm change similarly. The head is transformed into a vesicular male pronucleus accompanied by two centrosomes. Some of these pronuclei may fuse with the female pronucleus, so that giant polymorphic nuclei are formed by multipolar mitoses.

The isolated male pronuclei also divide and form haploidal bipolar mitotic figures of the usual structure. Mitotic division of the nuclei may continue, but the cytoplasm usually does not divide or cleavage is only partial and such embryos soon die.

4.6.4. Cyclostomi

Fertilization is external in this group and the gametic association occurs in water. The fairly dense egg membranes of lamprey lack micropyles and are permeable to spermatozoa in a limited region near the animal pole where there is a transparent jelly coat on the surface of the two-layered zona radiata (see Kupffer and Benecke, 1878; Kille, 1960a).

The lamprey egg is penetrated by a single spermatozoon. This was established in a series of investigations performed on different species (see Table 28). There are indications of the possibility of polyspermic fertilization, which was observed rarely only by Chubareva (1957a, b). Among more than two hundred eggs examined, two cases of dispermy (at pronuclei formation stage) were confirmed.

4.6.5. Amphibians

Among amphibians, fertilization was studied in detail for representatives of two orders, Caudata (Urodela) and Salientia (Anura). Hardely any data are available for Gymnophiona of two orders.

There are important differences in the method of fertilization among Urodela and Anura; the vast majority of urodeles are characterized by internal fertilization, while almost all anurans have external fertilization (Noble, 1931, 1954; Angel, 1947). They are also distinguished by features of the course of the fertilization itself. Therefore, the data referring to these groups will be examined separately.

The number of spermatozoa penetrating the amphibian egg can usually be ascertained by inspection of the egg, since a small recess forms and pigment concentrates at points where spermatozoa penetrate in cytoplasm. In eggs of species with fairly intense pigmentation, this spot becomes clear after a certain time as a dark spot with a light border.

As early as the 1870s, Van Bambeke, having observed such pigment patches or "vitelline pits" ("trous vitellins") formed in different amphibians newts, axolotls, frogs, toad frogs, and toads — and the pigment traces of sperm heads embedded deep in the ooplasm, concluded that fertilization is polyspermic in urodele species and usually monospermic in anurous species (Van Bambeke, 1870, 1876). This was subsequently confirmed.

Urodela. Among urodele amphibians are representatives of the most primitive families, Hynobiidae and Cryptobranchidae, in which the gametic association occurs in the external milieu, unlike other urodeles.

Under natural conditions fertilization in Hynobiidae is regularly monospermic, as was shown for Hynobius nebulosus (Kunitomo, 1910; Nakamura, 1933) and H. retardatus (Makino, 1934).

The pigment patch at the point of penetration of the spermatozoon into the ooplasm was determined for 116 eggs of Hynobius nebulosus (Kunitomo, 1910). The spermatozoa usually penetrated in the animal pole, but sometimes (10% of cases) the vegetal pole. In each of the eggs inspected there was only one such patch. Cytological investigations of subsequent stages of fertilization showed not a single case of polyspermy.

With artificial insemination of ripe (Nakamura, 1933) or just laid eggs of H. nebulosus (Kakiyama, 1934), fertilization is monospermic; however, if the eggs are kept in water for one hour before insemination, they are often penetrated by several spermatozoa (5–6 to 36; see Nakamura, 1933). Monospermic eggs develop normally, whereas polyspermic eggs cleave atypically.

Exceptionally, eggs of H. retardatus may be penetrated by two or more spermatozoa under natural conditions; in polyspermic eggs the female pronucleus fused with two male pronuclei and multipolar mitoses were observed at a later stage (Makino, 1934).

Hynobiidae are characterized by physiological monospermy, while polyspermy is distinctly pathological.

It is interesting that fertilization is often polyspermic when immature eggs of H. nebulosus taken from oviducts (at the end of the first maturation division) were inseminated, but the supernumerary spermatozoa did not behave like those in mature eggs. The heads of these spermatozoa did change into male pronuclei, but were not involved in development and gradually lyse as the synkaryon begins to cleave (Kakiyama, 1934).

It is very likely that a similar phenomenon was observed by Smith (1912) for Cryptobranchus alleghaniensis. The investigation was carried out on eggs taken from the lower sections of the oviduct and artificially inseminated. Judging by the pigment pattern on the egg surface, 1 to 10 or sometimes even more spermatozoa (maximum about 100) must have penetrated the eggs. In microscopic sections of eggs Smith found supernumerary spermatozoa only in the beginning of the fertilization process, while no more than two spermatozoa were found on transformation into male pronuclei (Smith did not indicate how often polyspermic eggs were encountered). After formation of pronuclei, supernumerary spermatic elements were no longer encountered. Comparing early resorption of the supernumerary spermatozoa with the normal development of most of eggs observed, Smith concluded that fertilization is physiologically polyspermic in Cryptobranchus alleghaniensis.

Eggs of C. alleghaniensis used for artificial insemination (at least some of them) were insufficiently mature. Smith demonstrated that the eggs are still not fertile in the initial period after descending into the lower sections of the oviduct. Somewhat later the eggs become fertile, but evidently not optimally so until laying when, under natural conditions, they are inseminated. To determine the type of fertilization in Cryptobranchus alleghaniensis it would be necessary to inseminate fully mature eggs or, better, to study eggs inseminated under natural conditions, as in the case of Hynobiidae.

Fertilization is internal in all the more highly organized urodeles (except for Sirenidae). The males have no copulatory organs. They discharge spermatophores, which the female captures with the edges of the cloaca.

Spermatozoa collect in the spermatheca – the cloacal pouch provided with tubular glands (homologous to the male pelvic gland). Spermatozoa in the spermatheca retain their fertilizing capacity for a long time: for example, about one year in Salamandra salamandra and perhaps even longer (Baylis, 1939). Gametic association usually occurs when eggs descend into the cloaca and pass by the spermatheca.

The eggs are covered with a thin vitelline membrane and strong jelly coats, permeable to spermatozoa which can penetrate at any point of the egg surface. In Diemictylus viridescens (Jordan, 1893; Fankhauser and Moore, 1941a), the spermatozoa penetrate the animal pole more often than the vegetal, as is the case in axolotls (Fick, 1893; Sládeček and Lanzová, 1959) or with equal frequency in all regions (Rott, 1967). In Triturus palmatus (Fankhauser, 1932), on the other hand, spermatozoa penetrate the vegetal pole more often than the animal pole.

The egg is generally penetrated by several spermatozoa (Table 29) without disrupting development in all urodeles with internal fertilization. Species with larger eggs are more intensely polyspermic (eggs of Ambystoma jeffersonianum and A. maculatum are normally penetrated by up to 8 spermatozoa, whereas Diemictylus viridescens eggs contain up to 19 and Triturus pyrrhogaster up to 32 spermatozoa).

As shown by cytological investigations on various urodele species (Fick, 1893; Jordan, 1893; Braus, 1895; Michaelis, 1897; Jenkinson, 1904; Bataillon, and Tchou-Su, 1929, 1930; Fankhauser, 1932; Fankhauser and Moore, 1941a; Rott, 1967, etc.) all the spermatozoa in the egg change similarly at the time of formation and approximation of the pronuclei. The sperm head swells, becomes vesicular and, usually, the seminal aster develops. These transformations proceed more rapidly in the vegetal than in the animal pole. The asters in the animal pole are considerably more developed than in the vegetal pole where they are generally smaller or even indiscernible; apparently, this is due to different amounts of hyaloplasm in these parts of the egg. Seminal asters in the animal pole repel one another, so that they are distributed more or less evenly (as with normal fertilization in selachians and experimental polyspermy in frogs).

The female pronucleus moving from the periphery of the egg into the cytoplasm unites with the male pronucleus which happens to be closest. From this moment, the supernumerary male pronuclei show increasing signs of degeneration.

In newts many of the pronuclei are ready for mitosis (with a delay compared to synkaryon); prophasic chromosomes of an atypical type appear, the seminal aster generally does not divide and monocentric mitosis is blocked. The ultimate stage of transformation of the male pronuclei is dissolution of the nucleic membrane and formation of a characteristic group of karyomeres; such groups were first described in eggs of Triturus alpestris by Braus (1895) who erroneously supposed that the chromosomal vesicles are formed from a mitotic division of the supernumerary male nuclei. Occasionally division of the seminal aster is observed, but no spindle is formed (Michaelis, 1897; Bataillon and Tchou-Su, 1930). Progressive development in the animal pole of the cleavage amphiaster drives the supernumerary male nuclei in the direction of the vegetal pole. During cleavage the degeneration nuclei are observed at the periphery of the vegetal blastomeres. Remains of these nuclei are visible until the 2—4-blastomere stage, sometimes until the blastula stage when they are completely lysed.

In Ambystoma jeffersonianum and A. maculatum the supernumerary male pronuclei rarely divide (Piersol, 1929), and in A. tigrinum never divide (Rott, 1967); in all these species the male nuclei degenerate before cleavage begins.

Urodele eggs artificially inseminated with a concentrated spermatozoan suspensions (Born, 1892; Fankhauser, 1925) or subjected to unfavorable conditions before fertilization (Piersol, 1929) often are penetrated by many more spermatozoa than normally characteristic for the particular species. Such extreme polyspermy disrupts development and causes early death of the eggs.

Cleavage of eggs of Triturus palmatus containing various numbers of penetrating spermatozoa was invariably disrupted if the number exceeded 10 (Fankhauser, 1925). Even this runs a whole gamut of transitions from extremely weak to sharp. For example, the presence of numerous supernumerary pronuclei and cytocentra may cause a slight deviation of the first cleavage furrow from its usual direction which is soon regulated. However, the first cleavage division may be accompanied by the formation of a large number of excess furrows due to the involvement of sets of supernumerary spermatic complexes in development (as with pathological polyspermy in normally monospermic eggs). These latter deviations lead to early cessation of development and to degeneration; if the number of spermatozoa exceeds 20, the eggs die without completing the first division.

Piersol (1929) noted intensive polyspermy in Ambystoma jeffersonianum under natural conditions when there was a sharp drop in temperature at the time of egg-laying. Cytological analysis showed that such eggs may be penetrated by up to 100 or even more spermatozoa (the maximum number observed was 368). A large percentage of such eggs cleave atypically, and form excess blastomeres each containing one or several male nuclei, which are preserved longer than in normally developing eggs. After the first division it is usually impossible to discern supernumerary nuclei, which were visible during several of the first cleavage divisions in intensive polyspermy. These nuclei then degenerated and the seminal asters also disappeared a little later. As a result, a considerable part of the egg occupied earlier by supernumerary spermatic complexes was excluded from development. Such eggs had blocked cleavage and died.

Thus, fertilization in the primitive Hynobiidae is monospermic, whereas in all higher urodeles polyspermy occurs regularly (without exception in all species studied and in a very high proportion of cases). At the intensity characteristic for each species, this polyspermy does not disrupt development.

Anura. Fertilization is external in most anurans (the sole exceptions are frogs of the genus Ascaphus and African toads of the genus Nectophrynoides).

As with urodeles, the eggs are covered by a thin vitelline membrane and a strong tertiary jelly coat, permeable to spermatozoa. Spermatozoa may penetrate the egg at any point on the surface of the animal pole in Rana (Hertwig, 1877; Herlant, 1911) and Bufo (King, 1901), but in Discoglossus pictus (Hibbard, 1928a, b) they penetrate in a more limited region close to the animal pole where there is a depression filled with a jelly plug (a strong local thickening of the egg membranes).

The anuran egg is normally penetrated by only one spermatozoon, as established for many species (see Table 28). However, polyspermy of varying intensity is observed in frog eggs on artificial insemination with highly concentrated sperm, or when overmature eggs are used. The development of polyspermic embryos of the common frog was analyzed in detail by Brachet (1910a, b, 1912) and his colleague Herlant (1911). These investigations are of great interest, since polyspermic eggs in frogs and in sturgeons develop very similarly.

In experimental polyspermy in Rana temporaria, the initial changes in the penetrating spermatozoa are exactly as in physiological polyspermy in urodeles. All the spermatozoa change synchronously, the heads are transformed into vesicular pronuclei of similar structure and seminal asters develop along with them. The seminal asters repel one another as seen from the difference in the pigmented spermatic traces; as a result the spermatic complexes — the nucleus with the centrosome and the surrounding cytoplasm permeated by its rays ("spermatic energid," according to Brachet) — are evenly distributed in the animal hemisphere. The female pronucleus, moving deep into the egg, enters one of the energids and fuses with the male pronucleus in it. From the moment of fusion, degeneration of the supernumerary spermatic complexes begins in urodele eggs; however, this does not occur in polyspermic frog eggs where all nuclear elements (synkaryon and haploid male pronuclei) begin mitosis synchronously.

In typical cases of weak and moderate polyspermy (i. e., when the number of spermatozoa does not exceed 10—13), division of the male nuclei proceeds normally. The bipolar mitoses terminate at the first cleavage division when the egg divides into as many blastomeres as spermatozoa which have penetrated it. In dispermic eggs, which are no exception to this rule, the spindles of the synkaryon and of the haploid mitotic figures attempt to align parallel to one another (in conformity with Hertwig's rule); such eggs divide into two blastomeres and are superficially indistinguishable from normal monospermic eggs. However, each blastomere of the dispermic egg contains two nuclei, so that at the second division such eggs divide into six blastomeres.

During cleavage, mitoses of the diploid nucleus (derivatives of the synkaryon) and haploid nuclei (descendants of the supernumerary spermatozoa) are synchronous and lead to formation of a more or less regular morula. In some blastomeres containing more than one nucleus, division is then blocked in the following manner. The mitotic figures unite and form multipolar mitoses not accompanied by cleavage. The approximated nuclei fuse and soon lose their capacity for division. The centrosomes continue for a time to divide independently and then degenerate. Embryos with such disruptions usually die without completing gastrulation.

Only a few embryos (10% among dispermic and trispermic ones, and only isolated embryos at a higher intensity of polyspermy) are capable of relatively prolonged development. Sooner or later, various defects are found. Polyspermic embryos survive for not more than 4—9 days; dispermic and trispermic embryos under the best conditions live for 1—3 months.

In intensive polyspermy where spermatozoa penetrate the egg close to one another, they form groups of 2–6. These male pronuclei fuse even before the development of seminal asters. At the first division stage, multipolar mitoses appear which do not lead to cleavage. Accordingly, more or less extensive regions of the egg remain undivided and become necrotic, so that the embryos degenerate in early stages of development without even commencing gastrulation. In cases of particularly intensive polyspermy (penetration of 60–100 or even more spermatozoa) the eggs are totally incapable of development.

Thus all polyspermic eggs are nonviable, including those from which embryos of a more or less normal type initially developed. Brachet considered this to be mainly due to retardation of cleavage connected with the formation of multipolar mitoses and the chimaeral structure of the embryo (organs of such embryos consist of a large mass of haploid cells whose nuclei were derived from various spermatozoa and are qualitatively different). Brachet also indicated the possible significance of quantitative deficiency of chromatin in the haploid cell elements.

It should be noted that no differences were observed in the mitotic activity of different spermatic complexes; they all displayed a capacity for division and under conditions of isolated arrangement produced bipolar mitoses which were completed normally.

From the above data it is clear that anuran fertilization is physiologically monospermic, and that experimental polyspermy is distinctly pathological. We shall now examine the question of the number of spermatozoa penetrating the egg of the higher vertebrates (Amniota). In all classes of this group – reptiles, birds and mammals – fertilization is internal and the membranes covering the egg at the moment of gametic encounter are permeable to spermatozoa.

4.6.6. Reptiles

The ooplasm regularly contained several, sometimes many supernumerary male pronuclei at fusion and first cleavage stages in all the reptilian species investigated. These included the slowworm Anguis fragilis (Opel, 1891, 1892; Nicolas, 1899, 1900, 1903, 1904), the green lizard Lacerta viridis (Oppel, 1891, 1892), the African lizards Camaeleon bitaeniatus, Camaesura anguinea, Mabuya megalura (Pasteels, 1956) and the grass snake Natrix (Tropidonotus) natrix (Oppel, 1891, 1892) and Vipera berus (Ballowitz, 1903a, b). The investigators did not succeed in observing subsequent stages of spermatozoan penetration and transformation. However, the youngest of the germinal discs contained some sperm heads at the stages of transformation into vesicular pronuclei, and some still in rod-shaped form (Oppel, 1891, 1892); unresorbed sperm tails were also found together with the pronuclei (Nicolas, 1900, 1903). There is thus no doubt that the extra nuclear elements originated from supernumerary spermatozoa.

In the slowworms, grass snake and viper, polyspermy may reach the same intensity as in selachians (see Table 29). In Anguis the number of spermatozoa penetrating the egg varies from 1–6 (Oppel, 1892; Nicolas, 1903) to 19–45 (Nicolas, 1900).

There is often a depression at the point where the spermatozoon penetrates the egg surface.

In the grass snake the structure of the supplementary male nuclei in the germinal discs varied considerably at the first cleavage division. Among the vesicular pronuclei with seminal asters were found compact round or rod-shaped nuclear formations without seminal asters, which are apparently sperm heads in the early stages of transformation. The very great differences in structure of male pronuclei may be due either to a prolonged period during which the spermatozoon penetrated the egg or else various rates of transformation of spermatozoa penetrating more or less simultaneously (Oppel, 1891, 1892). In the slowworm all the spermatic complexes have a similar structure; thus differences in time of penetration of the spermatozoa in this species cannot be very great.

During the first cleavage division the supplementary male pronuclei in reptiles, as in selachians, are pushed outward to the periphery of the germinal disc. Some of them, together with surrounding cytoplasm, are separated by accessory furrows. These furrows are usually not connected with one another or with the principal cleavage furrows initially dividing only the central part of the disc. The number of these furrows does not increase during the first divisions, and they disappear before the principal furrows reach areas where they were located.

At least some of the supernumerary pronuclei begin dividing (with a considerable delay compared to the synkaryon). The divisions consist of monocentric mitoses or of anastral mitoses with irregular arrangement of chromosomes along the spindle; such divisions are abortive and generally not completed.

The male nuclei show signs of degeneration at the 16-blastomere stage, and soon no traces of them remain (Oppel, 1892; Pasteels, 1956). Suggestions concerning the participation of these nuclei in the formation of a vitelline syncytium and construction of the embryo's tissues (Ballowitz, 1903; Nicolas, 1904) were not confirmed.

4.6.7. Birds

A brief summary of the literature on fertilization in birds was made by Lillie (see Hamilton, 1952) and Romanoff (1960).

Sperm penetration into hen and turkey eggs takes place soon after ovulation in the funnel of the oviduct (Patterson, 1910; Olsen, 1942; Olsen and Fraps, 1944; Olsen and Neher, 1948; Novik, 1957; Bekhtina, 1958). Harper (1904) observed spermatozoa in a state of transformation in pigeon eggs which had entered the funnel; he therefore assumed that the gametic association may occur before the egg reaches the oviduct, and possibly even before ovulation is completed when rupture of the follicle wall exposes the germinal disc.

Spermatozoa penetrate the egg in a limited region in the center of the germinal disc. This region has a width of 0.5 mm in a pigeon egg with a disc diameter of 3.5 mm (Harper, 1904).

Fertilization in birds is as a rule polyspermic, but the intensity of polyspermy may vary considerably; from 13 to 26 spermatozoa penetrate in the pigeon egg, and up to 29 (but usually only 3—7) in the hen's egg (Table 29). Polyspermy in birds is apparently not obligatory; a few of the swallows' eggs studied contained no supernumerary spermatozoa (Van Durme, 1914), while the monospermic hen's eggs occasionally encountered develop quite normally (Bekhtina, 1966).

The heads of all the spermatozoa penetrating the cytoplasm are synchronously transformed into pronuclei of similar structure. At the time of fusion of one pronucleus with the female pronucleus and during the first cleavage division, the supernumerary male pronuclei move to the edge of the germinal disc, and even beyond it into the coarsely granular yolk. The male pronuclei embedded deep in the egg (in the region of the "central periblast") rapidly degenerate. The remaining pronuclei, displaced peripherally, show several mitotic divisions, which are accompanied by the formation of superficial accessory furrows (as in selachians and reptiles).

In the pigeon egg, the phenomenon of "accessory cleavage" is distinctly pronounced. The first mitotic divisions of the haploid pronuclei proceed regularly; the spindle has typical structure and chromosomes are regularly arranged among the daughter nuclei. The divisions are accompanied by the formation of a large number of superficial furrows and the separation of small blastomeres, varying in shape and size, along the edge of the germinal disc. Accessory furrows appearing at the first division stage remain clearly discernible right up to the 8-blastomere stage (Harper, 1904). At this time degeneration of the male nuclei begins, and they disappear approximately at the 32-blastomere stage along with the accessory furrows. After the cell walls of the accessory blastomeres separating the marginal blastomeres of the germinal disc from the yolk atrophy, their nuclei pass into the periblast and organize it (Blount, 1909).

In other birds, the accessory furrows appear slightly later and in a smaller number (none are found in some species). In sparrow (Van Durme, 1914) and turkey (Olsen and Fraps, 1944) eggs, they were described only at the second division stage, in hen eggs (Patterson, 1910; Olsen, 1942) at the second and third division stages. Usually they appear as isolated shallow radial furrows at the border of the germinal disc or beyond its limits. All haploid nuclei lying beside such furrows and in the yolk degenerate.

Commencing with the 4- and 8-blastomere stages large-scale and almost synchronous fragmentation of the supernumerary male nuclei is observed in whole mount preparations of hen blastodiscs (Fofanova, 1964).

Only isolated nuclei in a state of advanced degeneration can be found at the 32-blastomere stage in the hen's egg. Ejection of nuclei from marginal blastomeres of the germinal disc into the periblast occurs at the eighth to ninth cleavage division stages, and up to this moment the periblast contains no nuclear elements during a considerable time (2—3 hours). From this example, it is quite obvious that supernumerary spermatozoa are not the source of nuclei of the vitelline syncytium in birds' eggs (Patterson, 1910).

4.6.8. Mammals

The most primitive mammals belonging to the order Monotremata (subclass Prototheria) are of particular interest with respect to polyspermy. Representatives of this group differ sharply in egg sizes from higher mammals. Definitive oocytes in Ornithorhynchus (Platypus) anatinus are 4.2–4.3 mm in diameter and 3.5–4.0 mm in Tachyglossus (Echidna) setosus (Flynn and Hill, 1939), whereas the egg size (without membranes) in placental mammals varies between 0.06 and 0.18 mm (Austin, 1961). The eggs of Monotremata are rich in yolk and resemble in structure the eggs of selachians, reptiles and birds.

Only a small number of investigations have been devoted to the fertilization process in **Monotremata** since it is extremely difficult to obtain data on the early stages of development of these animals.

Initially, Gatenby and Hill (1924) described a platypus egg at an early stage of the fertilization process with neither pronuclei nor cleavage nuclei. The egg surface contained three more or less separate nucleus-containing formations, which the authors regarded as polar bodies; the marginal area of the germinal disc contained 10 free nuclei, presumably supernumerary male nuclei. Since this interpretation agreed well with the earlier established occurrence of physiological polyspermy in large, yolk-rich eggs of other vertebrate groups (selachians, reptiles and birds), the authors concluded, on the basis of a single egg (showing patterns difficult to interpret), that fertilization in Monotremata is polyspermic. This conclusion was subsequently quoted in many reviews and handbooks.

However, reinvestigation (Flynn and Hill, 1939) of the same object on the basis of a deeper knowledge of the embryology of Monotremata indicated the original inaccuracy. The egg described was in fact at a much later stage than supposed; it had cleaved, but the greater part of the blastodisc was torn away when egg membranes were removed, and three of the remaining blastomeres were mistaken for polar bodies. The free nuclei observed in this egg were not supernumerary spermatic elements, but nuclei of the vitelline syncytium.

The accumulation of considerable material on the development of the echidna Tachyglossus setosus enabled Flynn and Hill to give a coherent picture of the fertilization and egg cleavage processes in this species. Cytological investigation showed that out of 12 eggs at early developmental stages (6 eggs with formed pronuclei and 6 at the first cleavage stage) not one contained supernumerary spermatozoa, male pronuclei or their descendants, although spermatozoa were always found embedded in the protein membrane.

The only undamaged platypus egg of similar development was of atypical structure. This egg was apparently at the third division stage. In the center of the blastodisc there were 8 usual blastomeres, while beside them there was a group of 13 relatively distinct cytoplasmic concentrations (accessory blastomeres) each containing one or several (2–5) nuclei. The nuclei of the accessory blastomeres were smaller than the normal cleavage nuclei and poorer in chromatin. The deviation from the normal course of the cleavage process in this egg was due, as the authors claim, to penetration by supernumerary spermatozoa (i.e., a case of pathological polyspermy).

The data presented thus indicate that fertilization in the Monotremata is physiologically monospermic.

In the **placental mammals** (subclass Theria, infraclass Eutheria) fertilization has been studied in a large number of species in considerable detail.

It was shown that the gametic encounter occurs in the upper section of the oviduct (ampulla of the Fallopian tube). At this time the eggs are covered by a thick transparent membrane, the zona pellucida (corresponding to the zona radiata in other animals), and also by the cumulus oophorus, a follicular membrane of cellular structure. The follicular cells are cemented together by mucoprotein. In most of the species studied, this mucoprotein is rapidly dissolved by the specific medium of the oviduct and the hyaluronidase in the sperm heads, and the follicular cells disperse, leaving the transparent membrane exposed. However, the exposure of the zona pellucida is not a necessary condition for fertilization. Observations on laboratory animals indicated that the spermatozoa can penetrate through the follicular membrane which is preserved in some species (cats and dogs) right up to the first cleavage divisions (see Austin and Bishop, 1957a; Austin, 1959).

In all the mammals studied the egg is regularly penetrated by only one spermatozoon; polyspermic eggs are rare. Individual polyspermic eggs have been described in a large number of investigations of various species. Table 29 mainly includes data from sufficiently ample cytological material to enable us to judge the frequency of polyspermic eggs. The data given refer to eggs fertilized soon after ovulation (at optimal maturity). It can be seen that the frequency of polyspermy in all the species studied generally does not exceed 2–3%.

Fertilization occurs long after ovulation in animals mated or artificially inseminated at the end of the oestrus period when eggs already show signs of overmaturity. The percentage of polyspermy at this time may rise considerably; there were 9% polyspermic eggs in rats, 16% in rabbits (Austin and Braden, 1953) and 11% in swine (Thibault, 1959). Apparently overmaturation is also responsible for the high percentage (10%) of swine eggs with three pronuclei observed earlier in the experiments of Pitkyanen (1955). Such eggs, however, may have occurred either as a result of dispermic fertilization or through suppression of the second maturation division often observed in swine when mating is delayed (Thibault, 1959).

The vast majority of polyspermic mammal eggs are dispermic. Penetration of more than two spermatozoa is extremely rare. Thus, in a large series of experiments on rats (with various relations between the moment of ovulation and time of mating), 109 out of 2,987 eggs studied, contained two spermatozoa, and only three eggs contained three spermatozoa each; no eggs were observed with higher numbers of spermatozoa (Austin and Braden, 1953).

Cytological changes were followed in dispermic eggs of rats during fertilization (see Austin, 1961). Spermatozoa penetrating the ooplasm transformed synchronously and produced two similar male pronuclei, each half the volume of the single male pronucleus in normal monospermic eggs. On completed development, the two male pronuclei approach the female pronucleus and, after dissolution of the nuclear membranes, form three similar groups of chromosomes in the center of the egg in the prometaphase of the first cleavage division.

The development of a large number of seminal asters was mentioned earlier in cases of pathological polyspermy in roundworms, segmented worms, echinoderms, sturgeons and anuran amphibians. Fusion of several male pronuclei with a female pronucleus produces multipolar mitoses, so that the cleavage process of such eggs is atypical from the very beginning. In contrast, one bipolar spindle of a normal type is regularly formed in polyspermic rat eggs. This peculiar feature served as one of a basis for the hypothesis advanced by Austin (1965) that the spermatozoan centrosomes do not directly form the mitotic apparatus of cleavage in mammals (unlike the vast majority of other animals).

Owing to the formation of normal mitotic apparatus, the first cleavage stages of polyspermic rat eggs proceed typically. An in vivo study of eggs at the first to third cleavage division stages showed that dispermic eggs, despite their triploid condition, had no disruption of development and were distinguishable from monospermic eggs only by the presence of two sperm tail sections in the cytoplasm (Austin and Braden, 1953). Such triploid rat embryos may be implanted, but their development is disrupted and they survive no longer than the middle of the gestation period (Piko and Bomsel-Helmreich, 1960).

The above data show that in all cases sufficiently studied, fertilization in mammals was physiologically monospermic.

4.7. MECHANISM PREVENTING SUPERNUMERARY SPERMATOZOA FROM PARTICIPATION IN KARYOGAMY AND FORMATION OF EMBRYONIC TISSUES

Supernumerary spermatozoa in physiologically polyspermic eggs are destined to be destroyed (on the condition that the intensity of polyspermy does not exceed the usual limit for the particular species). However, as we have seen, degeneration and lysis occur at different stages in different animals. In gastropods, the spermatozoa are resorbed at the very beginning of development, already before the sperm heads are transformed into male pronuclei. The same thing may happen in part with supernumerary spermatozoa in insects. In the remaining instances, all spermatozoa penetrating the ooplasm are transformed synchronously into male pronuclei with accompanying seminal aster. The male pronuclei begin dividing simultaneously with the synkaryon or after a short delay, but these mitoses is not completed in most cases. In urodele amphibians and reptiles, a normal bipolar spindle is not formed, and monocentric or anastral mitosis is blocked; a spindle is formed in insects and the oligochaete Eisenia foetida, but mitosis ceases at the metaphase. In birds, development of the supernumerary spermatic elements advances somewhat further; the spermatic complexes apparently may divide a second time, and degeneration only begins at the 8-blastomere stage. Derivatives of supernumerary spermatozoa survive longest in selachian eggs. Spermatic complexes form regular bipolar spindles during the two first mitotic cleavage cycles and division proceeds almost synchronously with those of the synkaryon and cleavage nuclei. After their later displacement outside the germinal disc, disruptions occur in the structure of the mitotic spindles, but the interkinetic nuclei derived from supernumerary spermatozoa hardly show signs of degeneration (at least until the blastocoele stage).

Elimination of supernumerary spermatozoa from development generally involves two stages: 1) prevention of fusion of more than one male pronucleus with the female pronucleus (i.e., to ensure the diploid constitution of the synkaryon), and 2) subsequent resorption, preventing participation of the haploid male nuclei in construction of the embryonic tissues. Such stages are absent only in some gastropods in which resorption occurs before the beginning of karyogamy.

Rückert's (1899) hypothesis on the nature of the mechanism active at the first stage has retained its significance up to the present time. According to this hypothesis, forces of repulsion develop between the cytocentra which prevent the approximation of the nuclei associated with them. Fusion of female and male pronuclei may be due to the fact that the egg loses its centrosome during oogenesis. The female pronucleus on entering the sphere of attraction of a spermatic complex (consisting of the male pronucleus and the seminal aster whose rays penetrate the surrounding cytoplasm) fuses with this male pronucleus, while other male pronuclei are kept at a certain distance by the forces of repulsion between their own seminal asters.

The mutual repulsion of spermatic complexes is directly connected with the properties of the surrounding cytoplasm. In selachians the repulsion appears when the spermatic complexes are in the germinal disc, but diminishes sharply or is lost completely when they are displaced into the yolk-rich region where the nuclei approach one another and multipolar mitoses appear.

Such forces of repulsion also develop in physiologically monospermic eggs subjected to experimental polyspermy. Here, too, supernumerary male pronuclei may remain isolated from the fusing pronuclei. This is always observed in anuran amphibians, as well as in some echinoderms and lancelets. However, it is usual in cases of pathological polyspermy in roundworms, segmented worms, sturgeons, mammals and often in echinoderms and lancelets, in which all or many of the male pronuclei fuse with the female pronucleus. Such fusion always (except for mammals) leads to the formation of multipolar mitoses and disrupted cleavage.

The phenomenon described is apparently similar in its mechanism to fusion of groups of male pronuclei observed in highly intense polyspermy in frogs (Brachet, 1910a, 1917) and tritons (Bataillon and Tchou-Su, 1929). If spermatozoa penetrate close together and male pronuclei are approximate when the seminal asters are not yet developed, fusion occurs easily; the same occurs if only one aster develops in a group of supernumerary male pronuclei. The spermatozoa are a considerable distance apart in less intense polyspermy in large frog eggs, and interaction between the spermatic complexes begins when asters are sufficiently well developed to prevent association.

Sturgeon eggs are larger than frog eggs, but the region of penetration is strictly localized and limited to the micropylar apertures; thus spermatozoa are close to one another from the moment of embedding in the cytoplasm until the seminal asters have developed.

Multiple fusion is also observed in eggs of other animals (roundworms, segmented worms, echinoderms, lancelets, mammals) because of the proximity of spermatozoa due to the small size of the entire egg and not merely of the area of spermatozoan penetration.

The question arises why the female pronucleus never fuses with several male pronuclei in physiologically polyspermic eggs, although cases have been observed where several spermatozoa penetrate close together in the area of the female pronucleus. This may be assumed to be averted by the earlier appearance of repulsive forces than in physiologically monospermic eggs, due to more rapid formation of the seminal aster or such changes in the cytoplasm ensuring effective repulsion at the earlier stages of development of the asters.

Hardly anything is known concerning the mechanism involved in inhibition of development and resorption of supernumerary spermatozoa. Several investigators have theorized on the nature of these mechanisms and attempted to prove their hypotheses experimentally.

On the basis of study of the fertilization process in the Chinese silkworm, Bataillon and Tchou-Su (1933) attempted to link the mitotic blocking of supernumerary spermatozoa with the abnormally low nucleus/plasma ratio in the cytoplasmic islets of haploid nuclei. According to their hypothesis, the primordial excess of cytoplasm in such islets causes cleavage to precede nucleic division. The process is disrupted and the centrosomes diverge drawing the cytoplasm along with them; the metaphasic spindle is exposed so that it is fated to degenerate. To prove this hypothesis, Bataillon and Tchou-Su attempted experimentally to interrupt the rhythm of cell and nucleic diversion in a cytoplasmic islet including the synkaryon; it was important to discover whether this disruption leads to blocking of mitosis at metaphase. Although first attempts were unsuccessful, the desired results were obtained later by Tchou-Su and Chang-Ko (1949, 1951). Freshly laid Chinese silkworm eggs treated with potassium cyanide (as respiration inhibitor) often show cessation of division of the synkaryon at metaphase. Moreover, the cytocentra, which are less sensitive to oxygen deficit, separate from the blocked mitotic figure and divide independently, exactly as in polyspermic silkworm eggs with supernumerary male elements (without any external influence). On this basis Tchou-Su and Chang-Ko modified the original hypothesis, maintaining that blocked mitoses of supernumerary spermatozoa is due to slight asphyxia which is aggravated by the abnormally low nucleus/plasma ratio.

The hypothesis of Bataillon, Tchou-Su and Chang-Ko seems skillful but artificial. At best, it might explain the blocking of changes in the supernumerary spermatozoa at the metaphase of the first division (as occurs in the silkworm). However, it cannot be of general significance. Indeed, how can asphyxia and a low nucleus/plasma ratio in cytoplasmic islets containing a haploid nucleus explain the blocked development of supernumerary spermatozoa at the most diverse stages of their transformation (for example, before transformation into male pronuclei nuclei in gastropods, or after completing several mitotic cycles in birds and selachians)? The early disruption of mitotic activity of the male pronuclei in urodele amphibians and reptiles with frequent anastral and monocentric mitoses does not conform with the hypothesis which is based on data that the cytocentra are sensitive to oxygen deficit.

Some light on this matter is shed by investigations on urodeles begun by Spemann and continued by Fankhauser and his colleagues.

Spemann (1914) tied the egg of Triturus vulgaris in half with a hair loop soon after fertilization. Both the hemisphere containing the female and male pronuclei and, often, the other hemisphere containing one or several supernumerary spermatozoa (demonstrated by spermatic traces) developed. The half with male pronuclei cleaved in most cases, and sometimes an entire embryo developed from such a hemisphere. Spemann concluded that the activity of the supernumerary spermatozoa is suppressed directly or indirectly by the egg nucleus or the synkaryon in the normally developing egg. The hemisphere lacking a female pronucleus was stimulated to develop not only on complete separation from the other half, but also on partial constriction of the egg. Apparently, the inhibition is not effective through the plasmic bridge connecting the two halves.

Spemann's experiments were repeated on a large scale on Triturus palmatus and T. alpestris by Fankhauser (1925, 1932, 1934), who also followed cytologically the behavior of spermatozoa under conditions of total or partial isolation from the synkaryon. If the diameter of the piece connecting the two halves exceeded half the original egg diameter, the development of the supernumerary spermatozoa was blocked as in an intact egg and all the male pronuclei degenerated rapidly at the stage of fusion. If, however, the septum was narrower, some of the spermatic complexes in the hemisphere lacking the female pronucleus showed a capacity for division; the wider the septum and the closer to the nucleus, the stronger the inhibition of the mitosis. These results served as a basis for a hypothesis modifying the original idea of Spemann. Accordingly, in normal development of the urodele polyspermic egg, the male pronucleus which fuses with the female pronucleus and, later, the synkaryon and the cleavage nuclei produce a certain chemical substance which diffuses in the cytoplasm and inhibits the activity of supernumerary spermatic complexes.

However, cytological study (Fankhaüser, 1934) of the development of the completely isolated egg hemisphere lacking a female pronucleus showed (that not only one inhibiting effect was responsible for the exclusion of supernumerary spermatozoa. In the absence of a female pronucleus and, consequently, of a synkaryon, some of the spermatozoa degenerate as rapidly as in the complete egg. This was later confirmed by experiments performed on Diemictylus (Triturus) viridescens (Kaylor, 1937; Fankhauser and Moore, 1941b) in which the female pronucleus was removed shortly after fertilization. In some eggs all the spermatic complexes degenerated as in the normally developing egg. In most eggs some complexes degenerated, while the others, capable of division, generally divided atypically with formation of multipolar spindles and random distribution of the chromosomes. In only a small percentage of cases did one of the male pronuclei divide normally like a diploid cleavage nucleus. According to the varied behavior of the male pronuclei, some eggs did not develop at all, most cleaved irregularly and chaotically and soon degenerated apparently due to the chromosome imbalance (see also Fankhauser, 1955). A few developed up to the later stages and produced androgenic larvae.

The behavior of spermatozoa in the absence of a synkaryon led Fankhauser to assume that two factors inhibited development of supernumerary spermatozoa in the case of normal fertilization. He believed that the urodele spermatozoa differ in their mitotic potency, some are incapable of normal mitosis without fusion with a female pronucleus and degenerate "automatically" after penetrating the egg. The more active and, therefore, more dangerous spermatic complexes are prevented from dividing by the inhibiting effect of the diploid mitotic system.

This explanation is not sufficiently well founded. It is improbable that a large proportion of urodele spermatozoa lost their capacity for independent mitosis, while all the spermatozoa retain such a capacity in other animals (echinoderms, selachians, sturgeons and anurans). It is more probable that the adaptation to physiological polyspermy in urodele amphibians is accompanied by certain changes in the ooplasm which inhibit the mitosis of the isolated spermatic complexes. In the other animals such a property did not arise since there was no need for it (it should be recalled that mitosis of supernumerary spermatozoa within the limits of the germinal disc is not suppressed in selachians; the other animals, echinoderms, sturgeons and anurans, are physiologically monospermic).

In physiologically polyspermic eggs the cytoplasmic inhibition of supernumerary spermatic complexes, apparently independent of the presence of a female pronucleus or synkaryon, also appears in androgenesis experiments where some or even all of the male pronuclei degenerate in the absence of a synkaryon. In the normal egg, the inhibiting effect may be increased after fusion of the pronuclei due to yet-unknown metabolic changes which diffuse from the locus of the synkaryon.

There are some data indicating the basis for development in physiologically polyspermic eggs of the capacity for eliminating supernumerary spermatozoa.

Bataillon and Tchou-Su studied the changes in immature oocytes of various animals (urodeles and anuran amphibians, Chinese silkworm) inseminated when already accessible to spermatozoa but still incapable of activation (Bataillon and Tchou-Su, 1928, 1930; Bataillon, 1929). Maturation division is not completed in such oocytes, and they show no superficial signs of development. However, cytological investigations in newt and silkworm eggs showed that all the spermatozoa penetrating the ooplasm began to divide, first forming monocentric, then anastral mitotic figures; the latter are usually bipolar and very closely resemble the polar spindle of the female pronucleus in shape. In the immature eggs, the transformations of the male pronuclei are blocked as with supernumerary male pronuclei in fertilized mature eggs. It is noteworthy that the same thing occurs with spermatozoa penetrating immature eggs of the physiologically monospermic anuran Hyla arborea, although supernumerary spermatozoa in its mature eggs may divide and develop along with the nucleic descendants of the synkaryon, which disrupts morphogenesis and kills the polyspermic embryos.

Polyspermic insemination of the physiologically monospermic eggs of Hynobius nebulosus at a stage when the still immature oocytes are already capable of activation is accompanied by early resorption of the supernumerary spermatozoa, which develop in mature eggs of this species and disrupt their growth (see pp. 216, 218).

It may be assumed from this discussion that the cytoplasmic properties of physiologically polyspermic eggs effecting elimination of supernumerary spermatozoa are not a new phylogenetic acquisition. They may have been retained during ontogeny from the cytoplasmic properties of immature eggs common to animals with different types of fertilization.

The mechanism for elimination of supernumerary spermatozoa from development, inherent in physiologically polyspermic eggs, has been discussed in this section. The protection of physiologically monospermic eggs from penetration by supernumerary spermatozoa (block to polyspermy) has a completely different basis and will be examined specially in Chapter 6.

4.8. CORRELATIONS BETWEEN THE FEATURES OF EGG STRUCTURES, CONDITIONS OF THE GAMETIC ENCOUNTER AND NUMBER OF SPERMATOZOA PENETRATING THE EGG ON FERTILIZATION

The survey of fertilization studies made in this chapter shows that monospermic fertilization is widespread in the animal kingdom. Physiological polyspermy has been established for representatives of three phyla: Arthropoda (insects), Mollusca (gastropods), and Chordata (selachians and chimaeras, urodele amphibians, reptiles and birds). Thus, physiological polyspermy is inherent only in a few groups of the more highly organized animals. It is thus likely that the original type of fertilization in animals was monospermic, and that physiological polyspermy developed secondarily during evolution. To determine possible causes for the appearance of polyspermy it is important to establish first whether correlations exist between type of fertilization, the egg morphology and the conditions under which the gametic encounter takes place in various animals.

As a result of early works carried out at the turn of the century revealing polyspermy in insects, selachians, reptiles and birds, the conviction developed that polyspermic fertilization is a feature of large eggs having a large yolk reserve.

A correlation between egg size and the number of penetrating spermatozoa was first noted by T. Boveri. He advanced the hypothesis that physiological polyspermy is an adaptation which in large eggs ensures association of the female pronucleus with the male pronucleus (Boveri, 1892, p. 401). Although Boveri's hypothesis was widely accepted, the fact, already mentioned by Rückert (1899), is that karyogamy is related not to the size of the entire egg but the size of the germinal disc, which is similar to that of many monospermic eggs.

The possible role of supernumerary spermatozoa in yolk resorption was also indicated as a biological value of polyspermic fertilization for large eggs. The transformation of supernumerary spermatozoan heads into nuclei of the vitelline syncytium, traced by Rückert for selachians, suggested that in multiple penetration of spermatozoa into large yolk-rich eggs serves to build the periblast, which assimilates nutritive substances from the yolk (Korschelt and Heider, 1903). It was subsequently shown, however, that the nuclei of the vitelline syncytium of holostean fishes, teleosts, reptiles and birds, like the syncytial tissue of the late mammalian trophoblast, originate from nuclei of peripheral cells of the germinal disc (see Nelson, 1953, pp. 321—322). Although the sperm nuclei are preserved longer in selachians than in reptiles and birds, it is difficult to imagine that in this group of animals their fate differs basically.

However, it is possible that supernumerary spermatozoa contribute enzymes and other substances aiding in assimilation of the yolk; their penetration is therefore of some benefit to large eggs (Nelsen, 1953).

At present, however, there is no basis for attributing to supernumerary spermatozoa any significant role in the morphogenesis and metabolism of embryos in yolk-rich eggs. This is indicated particularly by the large variations in the intensity of polyspermy. Physiologically polyspermic eggs may be penetrated by from one, several or a large number of spermatozoa; such variations are observed among different species of a single group (e.g., insects or birds) and also between different individuals of a single species (e.g., silkworm, hawk moth, bee, Pristiurus melanostomus, electric ray, axolotl, newt, slowworm, hen; see Table 29). Physiologically polyspermic eggs penetrated by only one spermatozoon develop quite normally.

The correlation between egg size and type of fertilization is not absolute. Physiological polyspermy has been observed in small eggs also (e.g., in gastropods). Furthermore, anuran amphibians are physiologically monospermic, but the vast majority of urodele amphibians are polyspermic, although their eggs do not differ sharply in size and yolk store. Although many teleosts have considerably larger eggs than those of urodeles, fertilization is monospermic in all teleost eggs.

The basic structure of the egg membranes has an important function in the gametic association; one might expect a definite correlation between permeability of the membranes and the type of fertilization, but there is none in fact. Physiological polyspermy is observed in eggs whose membranes are easily penetrated by spermatozoa (selachians, chimaeras, reptiles and birds), and in eggs with mostly sperm-impermeable membranes which have micropylar apertures (insects). It is significant that the insect egg can be penetrated by several spermatozoa not only when there are many micropyles, but also when there is only one (Drosophila).

On the other hand, monospermy is observed in many eggs with sperm-permeable surface membranes (e.g., in annellids, bivalve mollusks, echinoderms, mammals). Monospermy is observed in fish eggs having dense membranes both when there is one micropyle (teleosts) and when there are several (sturgeons).

The cortical layer of the egg is of prime importance and apparently determines the type of fertilization. Data on the structure of the cortical layer, given in Chapter 1, and on the type of fertilization in various animals, given here, show that fertilization is physiologically monospermic in all groups whose eggs have cortical granules or alveoli (Table 31). The structure of the cortical layer of the egg in many physiologically monospermic animals is not yet known, but it is very significant that no cortical granules or alveoli have been observed in eggs of physiologically polyspermic animals until now. Since in some kinds of eggs the cortical granules are very small and approach the limit of resolution of a light microscope, their absence can only be proven with an electron microscope. Such data are available at present only for Artemia salina (Anteunis and Fautrez-Firlefyn, 1961), two urodele species (Wartenburg and Schmidt, 1961; Wartenburg, 1962) and the hen (Bellairs, 1965, see p. 35).

TABLE 31. Presence of cortical granules or alveoli, location of gametic encounter and type of fertilization in eggs of different groups

Phylum	Class	Presence of cortical granules or alveoli	Type of fertilization	Location of fertilization
Porifera	Porifera	No data	Monospermy	Internal
Cnidaria		No data	Monospermy	Internal
Plathelminthes	Turbellaria	+	Monospermy	Internal
	Trematoda	No data	Monospermy	Internal
	Monogenoidea	No data	Monospermy	Internal
	Cestoda	No data	Polyspermy (?)	Internal
Nemathelminthes	Nematoda	+	Monospermy (?)	Internal
Nemertini		No data	Monospermy	External
Annelida	Polychaeta	+	Monospermy	External
	Oligochaeta	No data	Usually monospermy (in Eisenia facultative polyspermy?)	External (in mucous cocoon)
	Echiurida	No data	Monospermy	External
Arthropoda	Crustacea (Branchiopoda)	—	Facultative polyspermy (?)	Internal
	Crustacea (Amphipoda)	No data	Monospermy	External
	Arachnida	No data	Polyspermy	Internal
	Insecta	No data	Facultative polyspermy	Internal
Mollusca	Gastropoda	No data	Facultative polyspermy	Internal
	Bivalvia	+	Monospermy	External
Bryozoa		No data	Monospermy	Internal
Echinodermata	Crinoidea	+	No data	External
	Asteroidea	+	Monospermy	External
	Echinoidea	+	Monospermy	External
Chordata	Enteropneusta	+	Monospermy	External
	Ascidiae	No data	Monospermy	External
	Cephalochordata	+	Monospermy	External
	Cyclostomata	+	Monospermy	External
	Pisces (Elasmobranchii, Holocephala)	No data	Polyspermy	Internal
	Pisces (Teleostomi)	+	Monospermy	External
	Amphibia (Hynobiidae)	No data	Monospermy	External
	Amphibia (Caudata)	—	Polyspermy	Internal
	Amphibia (Salientia)	+	Monospermy	External
	Reptilia	No data	Polyspermy	Internal
	Aves	—	Polyspermy	Internal
	Mammalia	+	Monospermy	Internal

Finally the very considerable correlation between conditions of the gametic encounter and type of fertilization should be indicated. Fertilization is monospermic in all animals in which the gametic encounter is in the external medium (see Table 28). Polyspermy is encountered here in normal conditions very rarely in isolated eggs, and always involves disruption of development. Among these animals, the sole exception is Eisenia foetida in which development is apparently not disrupted when the intensity of polyspermy is very low. In this particular instance, however, the gametic encounter does not take place in marine or freshwater, but in a specific medium created by secretions of the dermal glands of the parent. Both physiologically monospermic and polyspermic forms are encountered among animals in which fertilization occurs in the genital tract of the female. (see Tables 29 and 31). It is significant that different conditions of gametic association within a single taxonomic group (phylum, class or even order) are associated with different types of fertilization. Among arthropods, fertilization takes place outside the genital tract of the female and is monospermic in amphipods, whereas it is internal and polyspermic in insects; apparently the same is true for the little-studied arachnids. Among mollusks, fertilization is external and monospermic in bivalves, but internal and polyspermic in gastropods. There are similar differences not only between urodele and anuran amphibians, but also among the urodeles the vast majority of which have internal and polyspermic fertilization except for the external and monospermic fertilization in representatives of the primitive family Hynobiidae.

To sum up, there is a correlation between the type of fertilization and of egg structure, primarily its cortical layer; eggs having cortical granules or alveoli show monospermic fertilization. There is also a definite relation between egg size and the number of penetrating spermatozoa, since polyspermy is observed mainly in large, yolk-rich eggs and a high proportion (with some exceptions) of large eggs are fertilized polyspermically. Finally, there is a correlation between the type of fertilization and the conditions of gametic encounter; eggs fertilized in the outer medium are physiologically monospermic, whereas there are both monospermic and polyspermic forms in eggs fertilized internally. The causes underlying these correlations shall be dealt with in Chapter 6, after the mechanism blocking polyspermy has been discussed.

Chapter 5

THE CORTICAL REACTION IN PHYSIOLOGICALLY MONOSPERMIC EGGS OF FISHES AND OTHER ANIMALS

Although the ooplasm of physiologically polyspermic eggs of various animals can be penetrated by several or many spermatozoa and the spermatozoa heads are generally transformed into male pronuclei, only one pronucleus fuses with the female pronucleus, while the remainder do not participate in karyogamy and degenerate sooner or later. In physiologically monospermic eggs, participation of only one spermatozoon in karyogamy is achieved in a different way. These eggs possess a special protective mechanism (block to polyspermy) which prevents penetration of supernumerary spermatozoa into the ooplasm after association with the first spermatozoon. This block evidently functions at the level of the egg surface — the cortical layer of cytoplasm and the membranes covering the egg. Therefore, changes occurring at the egg surface after contact of the spermatozoon and cytoplasm are described in this chapter in order to elucidate this mechanism.

5.1. DISCHARGE OF THE CONTENTS OF CORTICAL BODIES (GRANULES AND ALVEOLI) AND FORMATION OF THE PERIVITELLINE SPACE

Thorough cytological research revealed a layer of cortical bodies (granules or alveoli) distributed in the cortex (see p. 10) of mature unfertilized eggs of many physiologically monospermic animals. The cytoplasmic surface of the unfertilized egg adjoins the egg membrane, and they appear to be in close contact. In electron micrographs of such eggs, however, a narrow slitlike space is usually observed between the inner surface of the egg membrane and the cytoplasmic surface. This space, which we called the "primordial perivitelline space," intersects the microvilli emerging from the egg surface; the free ends of the microvilli penetrate the radial tubules of the egg membrane, sometimes through its entire thickness. Exceptionally, the mature unfertilized egg of sea urchins lacks the primordial perivitelline space, whereas eggs of some animals (e.g., mammals) right up to fertilization have a perivitelline space of considerable breadth, clearly visible in the light microscope (see below).

On activation, the cortical bodies are discharged from the cytoplasm to under the egg membrane, leading to a widening of the perivitelline space (or its formation in sea urchins). At the same time the microvilli retract. The egg is thus disconnected from the membranes and can orient itself within them according to its center of gravity.

The chain of initial changes in the cortical layer (cortical reaction) is usually initiated by contact of the spermatozoon with the ooplasmic surface, but may also be caused by various other activating agents.

Pasteels (1961) recently surveyed data on the cortical reaction in the eggs of various animals. With respect to the action of the mechanism blocking polyspermy, however, this survey is incomplete. Therefore, the course of the cortical reaction and its features in acipenserid and teleost eggs, as well as in eggs of other physiologically monospermic animals, will be examined below, partly reiterating Pasteels' information.

5.1.1. Acipenserids

The first changes observed in microscopic sections of fertilized eggs of Acipenser güldenstädti, A. stellatus or Huso huso involve discharge of the contents of the cortical granules. This process commences at the center of the animal pole where the micropylar canals are located; then the reaction spreads in all directions, involving consecutively the entire animal hemisphere, then the marginal zone and finally the vegetal pole.

This process begins almost immediately after the spermatozoon, deep in the micropylar canal, contacts the ooplasmic surface. It was demonstrated experimentally that in many eggs this contact occurs 1–2 seconds after insemination (see Figure 52, a, b). The first morphological changes in the cortical layer observed in some eggs with standard cytological methods within 3–4 seconds after insemination (at 18°C) involve the appearance of small vacuoles near the micropylar tubules and loss of the distinct contours of the granules. In subsequent seconds, the vacuoles enlarge so that the sections of cortical cytoplasm between them become column-shaped. As the vacuoles enlarge further, the columns are drawn out and separate from the egg. In some eggs, the cortical material at the animal pole separated within 5 seconds after insemination (Detlaf, 1957, 1961b, 1962). Such early release of cortical material was also described in sturgeon eggs activated by pricking with a glass needle (Ginzburg, 1960a; 1961a). The cortical material discharge then forms a thin layer under the inner vitelline membrane (Detlaf, 1957).

The changes in the cortical layer observed with the light microscope undoubtedly represent the onset of the cortical reaction. However, the representation of the morphology of the discharge of the cortical material is distorted and incomplete; in particular, the early casting off of the granule material is an artefact due to the cytological treatment of the egg. This was proven by ultramicroscopic observations of the cortical reaction in Acipenser stellatus and A. güldenstädti eggs (Ginzburg, unpublished).

A few seconds after the gametic encounter, the cortical granules open at the side toward the surface; 5 seconds after insemination the granule contents were observed issuing into the slitlike perivitelline space (compare Plate XI, a and b). The cytoplasm between the granules acquires a foamy structure (apparently as a result of hydration) and small granular elements and vesicles remain suspended in it. In the light microscope the foamy cytoplasm appears like vacuoles.

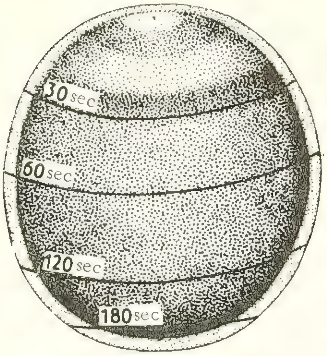

FIGURE 55. Rate of spread of cortical change in a sturgeon egg (after Detlaf, 1961b).

Each line corresponds to the boundary of the cortical reaction in seconds after insemination (18°C).

The discharge of the cortical granule material proceeds more slowly. Individual granule contours are still discernible after 30 seconds, although it can be clearly seen at this time that the granular membrane is entirely (not only on the surface side) dissolved (Plate XI, c). Even after 120 seconds (Plate XI, d), the discharge is far from complete; cytoplasmic extrusions derived from the strands between the granules protrude into the contents of the granules which fused into a single layer. Two components of the granule are clearly visible in the excreted mass — the compact dense inclusions and the lighter matrix. Neither dissociation of the secretion on the cytoplasmic surface nor noticeable liquidization of the secretion are observed. Evidently, the dissociation observed in standard histological treatments as early as 5—10 seconds after insemination is due to artificial detachment (probably related to decreased viscosity of the surface layer of cytoplasm after the beginning of the cortical reaction).

The cortical reaction spreads over the egg surface at an uneven rate, gradually decreasing in speed toward the vegetal pole. The boundary of visible change reaches the marginal zone 60 seconds after insemination (at 18°C), but encompasses the entire egg only after more than 180 seconds (Figure 55).

The membranes of the fertilized egg become sticky and adhere to the substrate after the cortical reaction ends at 2—5 minutes or more after insemination, depending on the temperature and specific features of the given batch of eggs. At the same time, the egg begins to change its orientation within the membrane, so that the animal pole gradually is uppermost (Detlaf and Ginzburg, 1954).

The change in orientation, also observed by Ancel and Vintemberger (1948) on activation of the frog's egg, occurs at a time when the perivitelline space is still so narrow (2—3μ) as to be indiscernible in live mounts at low magnifications in a vertical compartment to enable examination from the side.

In such mounts a narrow slit become visible between the cytoplasmic surface and animal pole membrane only when the change in orientation has been accomplished, 12—20 minutes after insemination. This slit gradually widens during the following 20—25 minutes, the animal pole is depressed and the perivitelline space reaches considerable size. This space is filled with a slightly turbid, faintly opalescent fluid. The contents of the perivitelline space coagulate and form into a dense gel in eggs fixed in 4% formalin (Detlaf and Ginzburg, 1954; Ginzburg and Detlaf, 1955; Zotin, 1961a). Cytological examinations indicate that the progressive enlargement of the perivitelline space is due to the excretion of a hydrophilic colloid from the lacunae of the animal pole (see p. 57).

Hydrophilic colloidal globules, appearing within the lacunae on fixation with Sanfelice fluid, rise during the first few minutes after insemination to the egg surface where they are hydrated and vacuolized. The hydrophilic colloids begin to pass into the perivitelline space 10–15 minutes after insemination, and are completely discharged within 40–50 minutes (Detlaf, 1961b, 1962).

The colloids discharged from the egg absorb water entering through the membrane from the surrounding medium (see Zotin, 1961a), thus forming the perivitelline fluid.

5.1.2. Teleosts

The contents of teleost cortical alveoli, like those of sturgeon cortical granules, begin to discharge at the animal pole, where the spermatozoon contacts the ooplasm in the single micropyle. Spreading over the egg surface and terminating at the vegetal pole, this process was first determined in cytological investigation of carp fertilization (Tschou-Su and Chen-Chao-hsi, 1936), followed in detail in live eggs of Oryzias latipes (T. Yamamoto, 1939, 1944a, 1962) and subsequently described in a number of other teleosts, such as chum salmon (K. Yamamoto, 1951b), ninespine stickleback (Kusa, 1953) and Fundulus (Kemp and Allen, 1956b; Armstrong and Child, 1965).

The membranes of Oryzias latipes eggs are sufficiently transparent to make them a convenient object for in vivo observation. If one observes an individual alveolus in any section of a fertilized or activated egg in the microscopic fluid, it may be seen at a certain moment how it decreases in diameter, becomes less and less discernible and then disappears completely. The blob of colloidal substance discharged from the alveolus increases in size and gradually spreads (Figure 56).

According to T. Yamamoto (1962), the entire alveolar membrane is dissolved in the cortical reaction due to esterase enzymes, presumably present in α-granules, arranged around the alveoli. Dissolution begins where the alveolus adjoins the egg membrane, forming an aperture through which alveolar contents pass outside. The alveolar membrane is dissolved by contraction of the alveolar cavity and its gradual displacement by surrounding cytoplasm.

Yamamoto's assumption concerning dissolution of the entire alveolar membrane agrees with results with sturgeons, in which electron micrographs showed lysis of the entire membrane surrounding the cortical granule.

With respect to the disappearance of teleost alveoli, ultramicroscopic study of the cortical reaction in Fundulus (Kemp and Allen, 1956b) and cytological investigation of lake trout eggs (Ginzburg, 1960b, 1961a) lead to

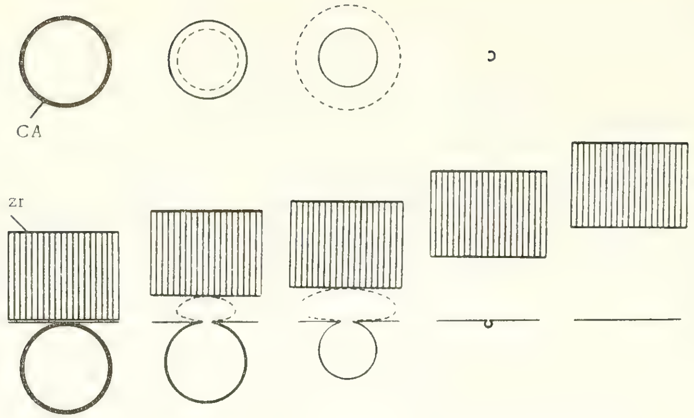

FIGURE 56. Discharge of the contents of a cortical alveolus in the egg of Oryzias latipes [schematic]:
Upper row — view from above; lower row — side view after (T. Yamamoto, 1962).

CA — cortical alveolus; zr — zona radiata.

rather different conclusions than those expressed by Yamamoto. Each cortical alveolus splits and in its place appears a crateriform pit. In trout eggs, a blob of alveolar colloids, coagulating on fixation, forms above the pit. While the aperture below the membrane gradually widens, cytoplasmic extrusions dividing the separate pits retract and the egg surface smooths out, the alveolar colloid is hydrated and spreads between the cytoplasm and zona radiata (Plate XII).

The wave of discharge of alveolar contents encompasses the entire egg surface within 2–3 minutes (3 minutes in Oryzias latipes at 23°C and 2 minutes at 28°C (T. Yamamoto, 1944a), in about 3 minutes in the ninespine stickleback at 18°C (Kusa, 1953).

The wavelike spread of the discharge of the alveolar contents is also observed in artificially activated eggs. However, the duration of the process depends on the kind of activating agent. In eggs of Oryzias latipes activated by chemical agents (sodium taurocholate,saponin and others) or increased temperature (T. Yamamoto, 1944a) and in eggs of chum salmon (K. Yamamoto, 1951b) and trout (Ginzburg, unpublished) treated with freshwater the cortical reaction begins exactly as in fertilized eggs near the animal pole (where sensitivity of the ooplasm is greatest) and terminates at the vegetal pole. When strictly localized effects are applied like needle pricks or direct current applied by an electrode the reaction begins at the point of application and spreads in waves over the egg surface (T. Yamamoto, 1944a). When the region of the animal or vegetal pole is pricked the reaction terminates at the opposite pole; if the egg is pricked in the marginal zone, the alveoli break down more rapidly in the animal pole and the reaction terminates not opposite the point of pricking but closer to the vegetal pole.

Discharge of alveolar colloids and formation of perivitelline fluid are accompanied by a slower bipolar differentiation of the egg. The cortical cytoplasm retracts toward the animal pole (due to its mass the blastodisc enlarges and becomes convex) and, in many cases, oil droplets fuse and migrate. The relation between these processes is shown schematically in Figure 57.

After completion of the cortical reaction, demersal teleost eggs generally become sticky and adhere to a substrate or one another. In all teleosts, the egg, freed of the membranes, completes the change of orientation at this time; in most eggs, the blastodisc turns upward, but in pelagic marine eggs the blastodisc is downward.

The perivitelline space next enlarges and reaches a considerable size in the vast majority of teleosts. Data on the formation of the perivitelline space and limitation of its size in teleost eggs have been summarized and thoroughly analysed by Zotin (1961a), who also conducted original investigations. His analysis shows that the perivitelline space in teleosts is formed by excretion of alveolar contents and inbibition of water from the medium and the egg itself. Thus, while the osmotically active substances of the perivitelline fluid in sturgeons originate from the cortical granules and from globules of hydrophilic colloid in the animal pole, all these substances in teleosts are located in the cortical alveoli.

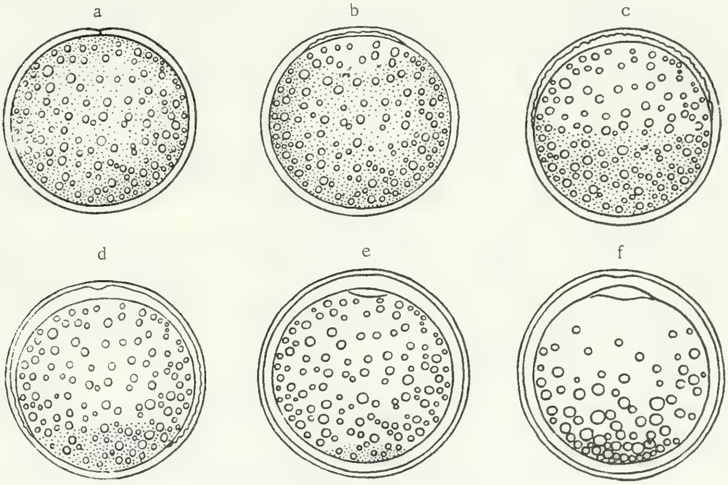

FIGURE 57. Cortical reaction and bipolar differentiation in the egg of Oryzias latipes [schematic]:

a—f — consecutive stages in excretion of alveolar contents (indicated by dots), migration of oil droplets (indicated by circles) and formation of blastodisc (after T. Yamamoto, 1961).

5.1.3. Sea Urchins

The initial changes in the fertilized egg have been investigated most thoroughly in sea urchins. Thus, a comparative survey of data on the cortical reaction should best begin with this group.

An excellent description of the cortical reaction in sea urchins (Echinoarachnius parma), based on microscopic observations of fertilization in living material, was given by Just (1919). The spermatozoon, having penetrated the jelly coat immediately after insemination, approaches the egg with rapid spiral movements. All movement ceases as soon as the tip of the head contacts the cortical layer. Then the sperm head is retracted passively into the egg. Soon after this, as the head is being completely absorbed in the cytoplasm and appears on the egg surface where it penetrated, formation of the perivitelline space and separation of the membrane begin. These processes spread in waves in all directions, but do not encompass the entire egg surface. Minute droplets discharged from the cortical layer are conveyed toward the receding membrane (these droplets are now known to be the discharged contents of one or more cortical granules). Some of these droplets are discrete bodies reaching the membranes, others are partially dissolved in the perivitelline fluid. Just noted that the membrane becomes somewhat thicker after separation.

Numerous later investigations made it possible to analyse separate stages of the cortical reaction. The results have been described in a series of reviews (see Runnström, 1952; Allen; 1958; Runnström et al., 1959; Pasteels, 1961, 1965a; Dorfman, 1963; Runnström, 1966). Only those results pertinent to the mechanism blocking polyspermy will be discussed here.

The unfertilized sea urchin egg is surrounded by a plasmalemma and a closely approximated, thin vitelline membrane. The boundary between these two membranes is invisible in a light microscope, and not always discernible even in electron micrographs. Though now conclusively proven (see Runnström, 1966), the existence of a vitelline membrane in sea urchin eggs was fiercely discussed for many years.

The two membranes on the egg surface form together numerous regularly distributed tubercles (Figure 5, b, Plate XIII, a). Immediately below the plasmalemma is the layer of cortical granules. Among and below these granules, the cytoplasm has normal structure and contains the same inclusions as in deeper layers.

The morphological changes in the surface of sea urchin eggs after fertilization have been followed in detail in the electron microscope (Afzelius, 1956a; Endo, 1961; Wolpert and Mercer, 1961; Lönning, 1967a). When the cortical reaction occurs in the region observed, the granule membrane splits where it adjoins the plasmalemma, and the contents of the granule are discharged. This is accompanied by separation and progressive retreat of the vitelline membrane from the egg surface (Plate XIII, b,).

The interpretation of Wolpert and Mercer, whose description of the cortical reaction in sea urchins has been used in Figure 58, deserves more detailed discussion. These investigators believed that the vitelline membrane always splits from the plasmalemma before uncovering the cortical granules. Such a sequence of events seems to them to provide a new understanding of the mechanism of discharge of the cortical granule contents. They assume that these granules are enclosed in pouches of the plasmalemma which are sealed by the vitelline membrane. Separation of the membranes leads to spontaneous bursting of the pouches and discharge of the granule material.

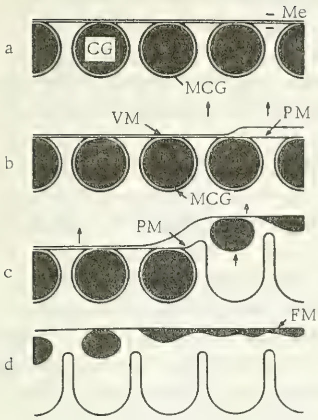

FIGURE 58. Processes occurring on the surface of a sea urchin egg in fertilization (schematic):

a — unfertilized egg; b — splitting of surface membrane into vitelline membrane and plasmalemma; c — uncovering of the cortical granules and discharge of contents; d — fusion of the contents of the cortical granules with the vitelline membrane to form fertilization membrane (after Wolpert and Mercer, 1961). VM — vitelline membrane; CG — cortical granule; Me — membrane on surface of unfertilized egg; MCG — membrane of cortical granule; FM — fertilization membrane; PM — plasmalemma.

Many data contradict this theory. Some of the cortical granules in the sea urchin, as in sturgeons, are not in contact with the plasmalemma (Lönning, 1967a). According to a number of investigators, several granules in the fertilized egg burst at various times after separation of the vitelline membrane and independently of this process. Breakdown of the vitelline membrane of the unfertilized egg by trypsin does not lead to discharge of the granule contents (see Runnström, 1966, p. 266). Thus, splitting off of the vitelline membrane where cortical granules have already burst (Wolpert and Mercer, 1961; see Plate XIII, b) is apparently not the cause but the result of this process. The discharge of osmotically active colloids is accompanied by inhibition of water from without and formation of perivitelline fluid, leading to pronounced tension of the vitelline membrane. It may be assumed that this also causes splitting off of the membrane on a small section along the boundary of the spread of the cortical reaction. With respect to the mechanism of bursting of the cortical granules, available data indicate that this process involves enzymes activated at fertilization (see Runnström, 1966, p. 272). .

After the granules have burst, small lumps of dense material present earlier in them (possibly related to lamellar structures; Endo, 1961) are revealed on the inner surface of the separated vitelline membrane (see Plate XIII, b, c,). The material derived from the cortical granules fuses with the vitelline membrane, transforming it into the "fertilization membrane."*

The cytoplasmic strands between the separate bursting granules are transformed at the egg surface into excrescences of irregular shape (see Plate XIII, c). Within several minutes after insemination, a structureless or fibrous material, most likely corresponding to the hyaline layer observed in the light microscope, is sometimes visible between these excrescences (Afzelius, 1965a). The contents of the cortical layer participate in the formation of this layer (Endo, 1961; Lönning, 1967a), as well as, apparently, substances discharged from the cytoplasm after secretion of the granule contents (Runnström and Immers, 1956).

Duration of cortical changes. Apparently there are no local differences in the rate of spread of the cortical reaction in the sea urchin egg; it proceeds evenly over the egg surface independent of the relation between the point of spermatozoon penetration and the animal-vegetal pole axis (not expressed morphologically in sea urchin eggs) and always terminates opposite to the point of initiation. Sea urchins differ in this respect from sturgeons and teleosts, whose cortical reaction spreading rate is much higher in the animal pole (which is probably connected with the distinct polarization of the egg cell).

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* Though still widely used, this term is archaic, since it expresses the out-of-date viewpoint that the unfertilized sea urchin egg, unlike those of many other animals such as annelids, mollusks and vertebrates, lacks a membrane and that the "fertilization membrane" is formed entirely of substances secreted by the fertilized egg (see Lillie, 1911, p. 361).

Having once begun, the discharge wave of the cortical granule contents encompasses the entire egg surface in Arbacia punctulata at 25.7°C in an average of 9.9 seconds (Moser, 1939), in Psammechinus miliaris at 20°C in 20–26 seconds (Allen and Griffin, 1958), and in Strongylocentrotus droebachiensis at 8°C in 90 seconds (Ginzburg, 1963c).

This wave was followed in the latter two species with great accuracy by interrupting the cortical reaction as proposed by Allen and Griffin (1958). Sea urchin eggs placed in 4% formalin at different times after insemination were immediately killed and the spread of the cortical reaction ceased, but the perivitelline space did form on that part of the surface where cortical granules had already burst. Thus, the boundaries of the reacted region could easily be determined at low magnifications (see Figure 59).

Comparison of these data with results of in vivo microscopic observations of fertilization in the same sea urchins show that separation of the membrane and formation of the perivitelline space where the spermatozoon penetrated become visible considerably after the cortical granules burst: after about 13 seconds in Psammechinus miliaris at 20°C (Allen and Griffin, 1958) and only after 30–40 seconds in Strongylocentrotus droebachiensis at 8°C (Ginzburg, 1963c).

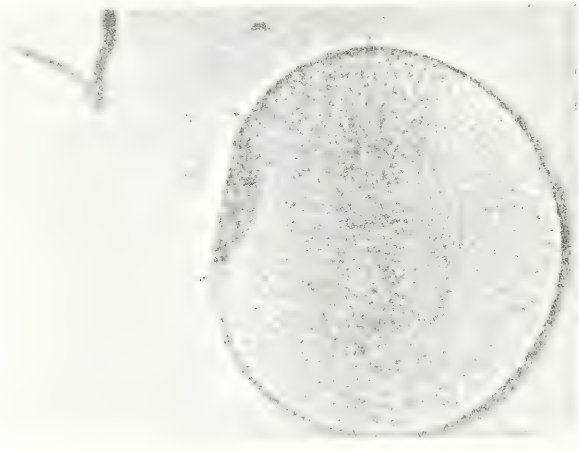

FIGURE 59. Egg of the sea urchin Psammechinus miliaris in which the cortical reaction was interrupted 24 seconds after insemination (20°C) by fixation in formalin (after Allen and Griffin, 1958).

The membrane has separated in the region where the cortical granules have already burst (boundaries indicated by arrows). FS — fertilizing spermatozoon.

The hyaline layer begins to form after discharge of the cortical granule contents, immediately after separation of the vitelline membrane (Moore, 1951; Hagström and Hagström, 1954b; Lönning, 1967a). Initially this layer is scarcely discernible, then it gradually thickens and is fully formed 15—20 minutes after insemination. Its thickness varies from a fraction of a micron to 2—3μ in different species (E. B. Harvey, 1956).

5.1.4. Invertebrates (except Sea Urchins)

The cortical reaction of fertilized eggs in other invertebrates has been studied relatively little. Cortical granules and alveoli have been described in representatives of five phyla of invertebrates — flatworms, roundworms, annelids, mollusks and echinoderms (see Table 1).

It has been shown for some turbellarians and nematodes that the cortical contents are discharged from the cytoplasm at fertilization (for references see p. 24). There are no data on the structure of the cortical layer in unfertilized trematode eggs but after penetration of the spermatozoon in Gigantocotyle bathycotyle separation of the membrane and formation of the perivitelline space were observed (Willmott, 1950). Carnot fixation reveals flakes of substances precipitated from the perivitelline fluid in this space.

Data on the cortical reaction in annelids and bivalve mollusks warrant more detailed examination.

Annelids. Among polychaetes there are considerable variations in the course of the cortical reaction.

In nereids, attachment of the spermatozoon stimulates the cortical reaction. Membranes surrounding the cortical alveoli fuse with the plasmalemma and with one another, the alveoli burst and their contents pass into the progressively widening perivitelline space (Fallon and Austin, 1967). Unlike sea urchin eggs, the vitelline membrane of nereid eggs is permeable to the discharged substances, which form a strong layer of a gelatinous substance on the outer surface of the vitelline membrane (as described for Nereis limbata by Lillie, 1911, 1919). Observations of the ultrastructure of the cortical reaction in eggs of N. diversicolor (Pasteels, 1966) show that all the cortical alveoli burst 30—60 seconds after insemination and the wide perivitelline space formed is filled with the alveolar contents and scraps of interalveolar cytoplasmic septa. Within 2 minutes, the cortical reaction is completed, a new cell membrane is formed on the surface of the ooplasm, and the alveolar contents pass through the vitelline membrane and form a gelatinous layer 6—7μ thick and penetrated by long cytoplasmic villi emerging from the egg.

The function of the cortical alveoli in the initial changes in the Nereis egg was demonstrated experimentally (Costello, 1949, 1958). Some cortical bodies are displaced in an unfertilized egg by centrifugation at 6,000—200,000 g, so that when such an egg is fertilized the perivitelline space at the point of displacement is wider and the jelly layer thicker. The greater the intensity and duration of centrifugation, the more pronounced the asymmetry. When centrifuged eggs are activated in alkaline NaCl solution, the discharged alveolar contents cannot pass through the affected vitelline membrane and swell the perivitelline space; the asymmetric distribution of this material leads to expulsion of the egg from the membrane in the region of centrifugal displacement.

The cortical reaction, like maturation, in Sabellaria and Pomatoceros is initiated by contact with seawater, independent of fertilization.

In vivo observations on Sabellaria vulgaris (Novikoff, 1939b) show that the cortical reaction begins immediately after the egg enters the seawater. Discharge of the cortical contents is accompanied by formation of the perivitelline space and partial separation of the membrane, which remains connected to the egg surface by cytoplasmic strands.

Fertilization of S. vulgaris eggs is possible from the moment they enter the water, before initiation of the cortical reaction, up to 5–6 hours after spawning. A fertilization cone is formed at the point of contact of the spermatozoon, with the participation of several cytoplasmic strands running from the egg surface through the perivitelline space to the membrane. When the cone together with the sperm head are drawn into the egg, all the cytoplasmic strands separate from the membrane and retract as well, completing the cortical reaction.

Electron micrographs of S. alveolata (Pasteels, 1965a, b) revealed that the cortical alveoli begin to swell no more than 4–5 seconds after the eggs are placed in seawater, and burst after 30 seconds. The alveolar contents issue from the egg, while interalveolar cytoplasmic lamina elongate and form a system of filaments, whose tips are embedded in the membrane. The spermatozoon penetrates at the locus of one such filament. According to Pasteels, fertilization causes no rapid change in the cortical layer. Within the following 10–20 minutes, only some of the cytoplasmic filaments retract, whereas some filaments, unlike what is observed in S. vulgaris, retain contact with the membrane.

The egg of the worm Pomatoceros triqueter behaves similarly (Hörstadius, 1923; Monroy, 1948). Mature unfertilized eggs undergo cortical changes in seawater, so that a perivitelline space forms, although the vitelline membrane remains connected to the egg surface by numerous cytoplasmic strands. The acrosomal filament of the spermatozoon probably contacts such a strand.

In Chaetopterus pergamentaceus, neither entry of the egg into water nor fertilization cause the discharge of the cortical alveoli, which remain in the surface cytoplasm of the fertilized and cleaved egg (Lillie, 1906; Rebhun, see Allen, 1958). However, it was demonstrated that the fertilized Chaetopterus egg secretes an osmotically active colloid (Henley, 1958); this process is accompanied by rhythmic waves of contraction of the vitelline membrane which begin approximately 20 minutes after insemination. Secretion of the colloid leads to formation of the perivitelline space and separation of the membrane. Some substances are discharged from the C. variopedatus egg, apparently within the first few minutes after gametic association when the intensity of the double refraction of the cortical layer distinctly decreases (like what occurs during spread of the cortical reaction in many sea urchin species). After this the volume of the egg decreases; the diameter of the unfertilized egg is 97.0μ, whereas 10 minutes after insemination it is 92.4μ (Monroy, 1953).

Apart from chaetopods, data on the cortical reaction are scant for other classes of annelids. It is known that in Nephelis (Hertwig, 1877) and in the echiurids Urechis caupo (Tyler, 1931), U. unicinctus (Sawada and Noda, 1963a) and Ikedosoma gogoshimense (Sawada and Noda, 1963b) fertilization is accompanied by separation of the membrane. Electron micrographs of U. unicinctus and I. gogoshimense (cited above) show that the primordial perivitelline space of the unfertilized egg, about 0.5μ in width, widens several times at fertilization. The cortical layer of mature eggs of these echiurid species lack cortical granules. However, almost-mature Ikedosoma eggs contain numerous large granules in the subcortical layer, which disappear during maturation. Evidently their contents pass into the cytoplasm. By analogy with the cortical reaction in eggs of polychaetes and many other animals, the widening of the perivitelline space at fertilization might be due to discharge of substances present earlier in these granules.

Bivalve mollusks. In most of species of bivalve mollusks studied — Mytilus edulis, Spisula solidissima, Barnea spinosa — fertilization is not accompanied by discharge of the cortical bodies' contents, as in Chaetopterus. Moreover, no other sharp changes were observed in the morphology of the cortex (Allen, 1953; Dan, 1962; Pasteels and de Harven, 1962; Rebhun, 1962; Humphreys, 1964).

Nevertheless, fertilization in some involves a very slight but indisputable increase in the size of the perivitelline space. The width of this space in Spisula solidissima eggs is, on average, 0.3μ, and after fertilization the width always increases no more than twice (Rebhun, 1962). After fertilization in Mytilus edulis the spaces widen between the microvilli and their cytoplasmic surface forms larger recesses as indicated by the increased volume of the perivitelline fluid (Dan, 1962) The slightest increase in the perivitelline space after fertilization has been established only in Barnea spinosa (Pasteels and de Harven, 1962).

In contrast, the contents of many cortical granules are discharged from the Mactra veneriformis egg at fertilization (accompanied by a considerable widening of the perivitelline space); nevertheless, part of them remain in the cytoplasm (Sawada and Muracami, 1959; Sawada, 1964).

Thus, the cortical reaction in Mactra is basically similar to that in the sea urchin and many other animals. As mentioned earlier, the cortical granules in the remaining bivalve species studied show a tendency to burst even before fertilization. Burst granules are also found in microscopic sections of eggs fixed at various times after fertilization. It may be assumed that the number of cortical granules (at least unchanged granules retained their contents entire) decreases during fertilization. The density of the cortical granules before and after fertilization was not determined.

If part of the contents of the bursting cortical granules is discharged at fertilization into the perivitelline space, a certain increase in its size must occur. The effect of a small volume of secretion possibly would not be discerned after fixation and dehydration of the eggs for ultramicroscopic investigation. The presence of free osmotically active substances in the mature unfertilized bivalve egg is confirmed by the swelling of the eggs when placed in seawater (the diameter of Ostrea gigas eggs increases from 54 to 60μ; Fujita, 1930).

Echinoderms. Apart from sea urchins, the cortical reaction has been studied only in starfishes; very little is known about other echinoderms. This process in starfishes is apparently very similar to that observed in sea urchins. In Asterina pectinifera, secretion of the cortical granule contents and separation of the membrane proceed exactly (with respect to morphology and rate) as in the sea urchin Strongylocentrotus pulcherrimus (Motomura, 1941).

5.1.5. Chordates (except Fishes)

Enteropneuste. The changes occurring in the fertilized Saccoglossus kowalevskii egg coincide in many respects with those observed in the sea urchin (Colwin and Colwin, 1949, 1954a; Colwin et al., 1956). The contents of the cortical granules discharge in the first few seconds after the spermatozoon attaches; this is accompanied by detachment from the egg surface of the two transparent membranes covering it; this process spreads in waves. The discharged blobs of granular contents swell strongly, fuse and unite with the inner membrane, which thus thickens. Granule material is added to the membrane also at another stage. Somewhat later a layer containing large globules of irregular shape appears on the egg surface. The Colwins believe that this layer is formed both by cortical granule material and substances secreted by the egg after all the granules are discharged. This layer tightly envelops the blastomeres during cleavage, and can be compared with the hyaline layer in sea urchin eggs. The space between it and the inner membrane is filled with a substance of gelatinous consistency. The considerable viscosity of the contents of the perivitelline space was noted also in another representative of this genus, S. horsti (Burdon-Jones, 1952).

Tunicates. Comparison of the early changes in the ascidian egg with those occurring in other animals is complicated by the peculiar structure of its coverings. The membrane of the mature ascidian egg is surrounded by a follicular layer. Between this membrane and the ooplasmic surface there is a second, inner follicular layer formed by special test cells. Soon after the eggs are spawned in the water, the membrane separates from the inner follicular layer and a large space appears between them; the test cells remain on the egg surface (Berrill, 1930; Monroy, 1953). The mechanism of formation of this space (incorrectly called perivitelline, since it is not homologous to the perivitelline space in eggs of other animals) is unknown. Berrill guessed that it arises due to the inhibition of water by the colloids, which are located between the chorion and inner follicular layer even before the eggs are laid. Nothing is known concerning changes in the cortical layer egg at fertilization (in particular, whether the contents of the vesicles under the cell membrane are discharged; see p. 29).

Acrania. Lancelet eggs have cortical alveoli containing small spherical bodies. Immediately after fertilization the alveolar contents issue from the cytoplasm, and spherical bodies are often discernible also in the perivitelline space (Sobotta, 1897). These bodies soon unite to form a homogeneous layer ("Hauptmembran", according to Sobotta's terminology) adjoining the much thinner egg membrane, which separates from the cytoplasmic surface after discharge of the alveolar contents. The formation of the membrane in the fertilized lancelet egg is similar in general outlines and in many details to transformation of the vitelline membrane into a "fertilization membrane" in the sea urchin.

Cyclostomi. Cortical changes in the fertilized lamprey egg have been studied quite fully (Herfort, 1901; Okkelberg, 1914; T. Yamamoto, 1944c; Kille, 1960a, 1961). Discharge of the contents of the cortical alveoli begins around the animal pole (since there are no cortical alveoli in the center of the pole, where the spermatozoon penetrates) and, as in sturgeons and teleosts, spreads in waves over the egg surface to terminate at the vegetal pole. The perivitelline space arises in the same sequence.

T. Yamamoto, who observed the changes in a living fertilized egg of the Far Eastern species of Lampetra reissneri (= L. planeri Regan), traced the discharge from the cortical layer of small clots of a transparent substance, which gradually spreads to form the perivitelline fluid.

The cortical reaction spreads more rapidly in the animal than in the vegetal pole (T. Yamamoto, 1944c), and encompasses the entire egg surface in L. fluviatilis and L. planeri within 16 minutes at 10°C or 9 minutes at 18°C (Kille, 1961). Observations on L. reissneri (Kusa and Ootake, 1959) indicate that the lamprey egg apparently continues to secrete some substances into the perivitelline space after discharge of the cortical contents. Soon after separation of the membrane, a large number of small bodies differing in appearance from the discharged cortical contents are found in the perivitelline space near the animal pole. These bodies are distributed in the perivitelline space and form a continuous layer around the egg; later this layer becomes indiscernible.

Urodele amphibians. In urodele eggs, fertilization is always accompanied by discharge of the cortical bodies' contents (Motomura, 1952; Katagiri, 1959; Osanai, 1960a; Wartenberg and Schmidt, 1961; Kemp and Istock, 1964, 1967). This process begins soon after activation of the cortical reaction; Rana pipiens eggs begin discharging the cortical granule contents 45 seconds after activation by pricking; the cortical reaction wave encompasses the entire egg surface within $1-1\frac{1}{2}$ minutes (Kemp and Istock, 1967). On insemination it takes the spermatozoon a certain time to penetrate the egg membranes; accordingly, granule contents of fertilized urodele eggs are secreted only 5–10 minutes after insemination (Osanai, 1960a; Kemp and Istock, 1967). Ten minutes in Rana nigromaculata (Motomura, 1952) and 15 minutes in R. temporaria after insemination (Wartenberg and Schmidt, 1961) the secretion process is close to completion, and the cortical granule contents are already in the perivitelline space where they swell and unite into a single mass. The colloidal interlayer between the cytoplasmic surface and the membrane, invisible in a light microscope, is sufficient (as in the case of sturgeons) for the egg to change its orientation. When eggs of Rana temporaria are activated electrically the orientation change begins 10–15 minutes and ends 30 minutes after the application of current, but the perivitelline space can be discerned in a horizontal light microscope only after 35–40 minutes (Ancel and Vintemberger, 1948).

The perivitelline space then widens considerably. Its increased size at the end of the cortical reaction is due to secretion of osmotically active substances from the animal pole (Hertwig, 1877; Bialaszewicz, 1912; Wintrebert, 1933; Detlaf, 1961b, 1962) in a process very similar to the discharge of hydrophilic colloidal globules from the animal pole of sturgeon eggs.

Mammals. Contents of cortical granules found in eggs of some mammalian species are discharged from the cytoplasm at fertilization (Austin, 1956a; Szollosi, 1962; Hadek, 1965). As in other animals, the secretion is stimulated by the gametic contact. Almost all the granules were discharged from eggs of golden hamster fixed with the spermatozoon just touching the cytoplasmic surface.

In hamster, rat and mouse eggs, the perivitelline space is clearly discernible before fertilization (the ultrastructure of its formation in the rat egg studied was traced by Odor, 1960); after gametic association its volume increases markedly (Austin and Braden, 1956).

5.2. LATENT PERIOD OF CORTICAL REACTION

The contents of cortical granules or alveoli do not begin to discharge immediately upon attachment of the spermatozoon to the ooplasmic surface, but after a certain time which differs in different animals. The period from the moment when gametic contact is established to the beginning of the discharge is called the latent period of the cortical reaction (Allen and Griffin, 1958; Allen, 1958).

Although this term is widespread, Pasteels (1961, 1965b) considers it unsuitable since the secretion of cortical contents is not identical with the cortical reaction but only a stage of the reaction, and is preceded by certain other changes in the cortex.

Although Pasteels' comment is correct, it is still possible to use the term proposed by Allen and Griffin (instead of the more accurate, but unwieldy term "the latent period of the process of secretion of the contents of the cortical bodies"), if one remembers the actual nature of the process.

The duration of this period can be determined in fertilized eggs, as well as in artificially activated eggs, which present certain difficulties in determining the commencement. In eggs activated by needle pricking, the initial moment is the time when the artificial stimulus is applied. However, since the effect of certain chemical agents does not appear immediately upon exposure to the chemical, it seems more correct to reckon the commencement of the latent period of the cortical reaction from the time when the activation impulse appears in the egg (see p. 261). It should be remembered that with such a criterion the duration of the latent period may be somewhat reduced.

5.2.1. Fishes and Cyclostomi

In fishes (acipenserids and teleosts) and lampreys, the latent period of the cortical reaction was determined by a single method. The eggs were activated with a needle, and the beginning of discharge of cortical contents was observed in vivo close to the point where the needle was applied.

In acipenserids, this experiment could be performed by using a batch of eggs with strongly pigmented animal poles. In such eggs the onset and spread of the cortical secretion can be clearly seen at low magnification (Ginzburg, 1960a, 1961a). The eggs were pricked near the animal pole. No changes were noted in the first 1–2 seconds. In the second or third second (at 21–22°C), a light-colored spot appeared at the point where the egg was pricked and rapidly increased in size and spread in all directions. This spreading was not fully symmetrical; the edge of the spot closer to the center of the animal pole moved more rapidly than the opposite edge. In some sturgeon eggs the light spot appeared 4–5 seconds or exceptionally even 10 seconds after pricking.

These observations, which agree with cytological and ultramicroscopic results, show that the latent period in acipenserids is very short, being generally on the order of 1–2 seconds.

The duration of the latent period may vary considerably in eggs obtained from a single female. Such variability is undoubtedly connected with the different physiological condition of these eggs; the varying quality of eggs within each batch of sturgeon eggs, obtained by dissection of a hypophysis-treated female, was revealed earlier during tests of their fertility and activation (Detlaf and Ginzburg, 1951).

When eggs of Oryzias latipes (T. Yamamoto, 1944b) and Lampetra reissneri (T. Yamamoto, 1944c) were pricked, the cortical secretion began much later than in sturgeon eggs in the region of the animal pole after 10–38 seconds at various temperatures (Table 32). If data obtained at a given temperature are compared, the difference is very clear: at 21–22°C the latent period in the sturgeon lasts 1–2 seconds and in Oryzias latipes 18 seconds.

TABLE 32. Duration of latent period of the cortical reaction (in seconds) of eggs of Oryzias latipes and Lampetra reissneri activated by pricking with a glass needle (after T. Yamamoto, 1944b, c)

Location of pricking	Oryzias latipes		Lampetra reissneri (L. planeri Regan)
	21–23°	28–30°	17–18°
Animal pole	18 ± 0.6	10 ± 0.2	38 ± 1.5
Marginal zone	26 ± 0.9	—	—
Vegetal pole	37 ± 0.8	22 ± 0.3	89 ± 4.2

The duration of the latent period of the cortical reaction in Salmo trutta m. lacustris activated experimentally by freshwater is about the same as in Oryzias latipes (Ginzburg, 1963a, b); the discharge of the cortical alveoli near the animal pole takes place 50–70 seconds after eggs are placed in water (at 8.9 and 3.0°C) or 30–50 seconds after appearance of activation impulse (see p. 263).

The duration of the latent period of the cortical reaction in fertilized eggs is known for Lampetra fluviatilis and L. planeri (Kille, 1961). The discharge of the contents of the cortical alveoli began 10 seconds (at 20°C), 12 seconds (18°C) and 20 seconds (10°C) after gametic contact. These data differ substantially from those obtained for artifically activated L. reissneri eggs (see Table 32).

It is hardly likely that species variations cause such great differences in the duration of the latent period at a given temperature (17–18°C) — 38 seconds in eggs of L. reissneri activated by pricking and 12 seconds in fertilized eggs of L. fluviatilis and L. planeri. These differences may be assumed to be connected also to the method of activation; the egg might react more quickly to contact with the spermic acrosome than to an artificial stimulus, such as pricking.

5.2.2. Sea Urchins

An approximate idea of the duration of the latent period of the cortical reaction in sea urchin eggs can be obtained from the data of Moser (1939), who showed that cortical granules breakdown where the spermatozoon is attached in the egg of Arbacia punctulata begins 10–20 seconds after insemination. However, it is not sufficient to know the time of insemination to determine when the latent period commenced, but the moment of actual gametic association must be established.

The first determination of duration of the latent period in sea urchins was made by Allen and Griffin (1958), who established the time of gametic association by using the method (Hagström and Hagström, 1954a) for inactivating free spermatozoa with detergent (sodium lauryl sulfate) at various times after insemination. The beginning of the cortical reaction was determined by placing the eggs at different times after insemination in 4% formalin (see p. 242). It was found that the latent period at 20°C lasted, on the average, 17 seconds in Psammechinus miliaris (from 12 to 22.6 seconds in different batches) and up to 40 seconds at 10°C. In eggs of Arbacia punctulata at 22°C the latent period was 17–23 seconds.

Similar results were obtained in ultrastructural study of the cortical reaction in P. miliaris (Wolpert and Mercer, 1961). Eggs fixed 30 seconds after insemination showed no changes in their cortical layer; after 40 seconds initial patterns of secretion of the cortical granule contents were observed (20°C). The experiment with inactivation of the spermatozoa by detergent showed that gametic contact in this series was established 10 seconds after insemination in 89% of cases. Thus, the latent period of the cortical reaction in the batch lasted between 20 to 30 seconds.

The latent period lasts correspondingly longer in eggs of Strongylocentrotus drobachiensis developing at a lower temperature (8°C) (Ginzburg, 1963c). In 7 series with eggs fixed in formalin at various times after insemination, it was established that the cortical discharge begins after 50–60 seconds in a few eggs, 70–80 seconds in most eggs and 90–140 seconds after insemination in all eggs of a particular batch. At the sperm concentrations used in these series (about 10^7 spermatozoa per cc), all the eggs were contacted by spermatozoa within 10 seconds (as shown by experimental inactivation of swimming spermatozoa by 0.06% formalin). Thus, the latent period of the cortical reaction lasts no less than 40 seconds and in most eggs 60–70 seconds.

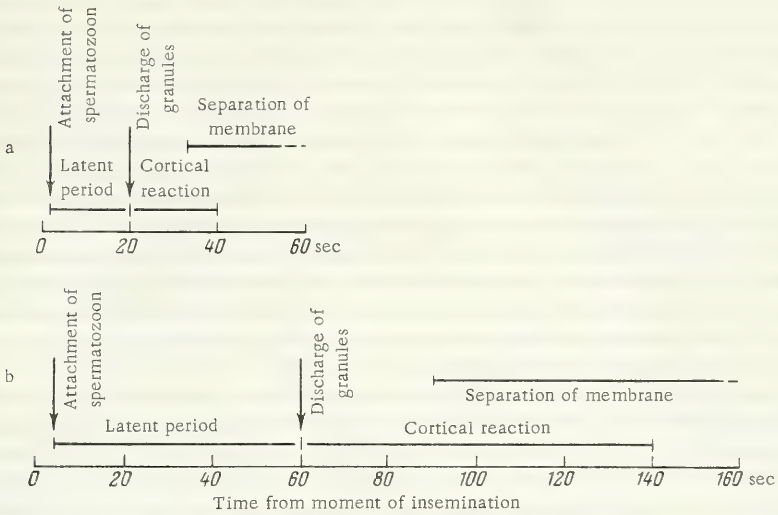

FIGURE 60. Timing of cortical changes in fertilized sea urchin eggs:

a — Psammechinus miliaris (20°C, after Allen and Griffin); b — Strongylocentrotus droebachiensis (8°C, Ginzburg, unpublished).

Comparing the duration of consecutive phases of the cortical reaction (Figure 60), it appears that the latent period in the sea urchin egg lasts approximately as long as the active phase (spread of the cortical secretion process over the egg surface). The membrane begins separating from the egg surface, observed in vivo, about the middle of the active phase.

There are no data on duration of the latent period of the cortical reaction for animals other than sea urchins, Cyclostomi and fishes.

5.3. CONCLUSIONS

The data presented above indicate a close similarity in the initial changes in fertilized eggs of physiologically monospermic animals belonging to systematically remote groups.

In typical cases studied well in echinoderms and chordates, contact between the spermatozoon and the ooplasmic surface stimulates the cortical reaction, which proceeds in extremely distinctive ways in different forms. After a certain latent period in the region of attachment of the spermatozoon, the cortical bodies burst and pour out their polysaccharide-rich contents under the membrane, the secretion wave spreads in all directions and gradually encompasses the entire egg surface. In many cases (in Cyclostomi, acipenserids, urodele amphibians, and, apparently, also in sea urchins and enteropneusts) a substance of different origin is discharged from the egg after the cortical reaction. These processes result in the separation of the ooplasm and egg membrane by perivitelline fluid containing egg secretions.

In eggs of other physiologically monospermic animals having cortical bodies, initial changes in the fertilized egg have been poorly studied. There are grounds for believing that a similar process exists in the case of such forms as turbellarians, and some roundworms and annelids. The deviations from the usual sequence observed in the polychaetes Sabellaria and Pomatoceros are not radical. In these worms the cortical reaction can be stimulated simply by contact with water, and not only by fertilization. However, only the initial stage of the reaction takes place; if fertilization does not occur, the ooplasm and the egg membrane do not separate but remain connected by cytoplasmic strands, which sense the contact with the spermatozoon. The changes in these polychaete eggs are in fact no different from those occurring in teleost eggs activated by water. Cortical alveoli burst, but up to a given moment (if the membrane and cytoplasm touch in the micropylar region) the spermatozoon can still be sensed (R. Suzuki, 1959a; Gamo et al., 1960).

Deviant forms include some species of bivalve mollusks and, among the polychaetes, Chaetopterus in which fertilization is not accompanied by a wavelike discharge of cortical bodies (similar in structure, position and chemical nature to the cortical bodies of other animals). In these cases, however, there are data on secretion of an osmotically active colloid whose origin has not been determined. A thorough study of the changes in the eggs of these animals would be of great interest.

Finally, there are no data on the fine structure of the cortical layer of the mature egg and its transformations during fertilization for many groups of physiologically monospermic animals. It is most likely that as the number of objects investigated increases in the future, cortical bodies and a cortical reaction like that in echinoderms and many chordates will be discovered in many more animals.

Having dealt with changes in the cortical layer of the egg during fertilization, we can now deal with the mechanism of the block to polyspermy.

Chapter 6

THE MECHANISM OF THE BLOCK TO POLYSPERMY

In the first investigations of fertilization in sea urchins and starfishes, Fol established that after the membrane separates from the egg surface (formation of the fertilization membrane, as it was regarded at the time) the egg becomes impermeable to spermatozoa. On the other hand, in those cases where the membrane separates more slowly than usual (immature, overmature or deficient eggs), polyspermy is often observed (Fol, 1877a, c, d; 1877–1879). Proceeding from these observations, Fol suggested that the fertilization membrane forms as a means to prevent polyspermy. Agreeing with this view, Hertwig and Hertwig (1877) showed that retardation of the cortical reaction in fertilized eggs by means of narcotics or other agents led to multiple penetration by spermatozoa.

However, separation of the membrane takes place a considerable time after insemination, when some of the spermatozoa usually have penetrated the egg surface. Thus, it appeared that the mechanism preventing polyspermy must operate at an earlier stage (Lillie, 1919, p. 162). This was conclusively proven by the detailed observations of fertilization in the sea urchin Echinarachnius parma made by Just (1919).

Just (1919) established that when the tip of the sperm head first penetrates the cytoplasm the egg surface in the immediate vicinity of this point becomes inaccessible to other spermatozoa. Those spermatozoa in contact with the egg in this region are removed together with the membrane when the perivitelline space is formed. Penetration of supernumerary spermatozoa in the rest of the surface is still possible. As the sperm head penetrates into the cytoplasm, cortical changes rendering its surface impermeable to spermatozoa spread further over the surface, so that only the opposite pole is permeable to sperm by the time the sperm head is completely embedded in the cytoplasm. Cortical changes already encompass the entire egg surface by the time separation of the membrane commencing at the point of spermatozoan contact is observed in the microscope.

Separation of the membrane results from earlier changes in the egg cortex – the cortical reaction, a complex multistage process lasting a considerable time (see Chapter 5). The question naturally arises as to which stage or stages are responsible for the block to polyspermy.

Initial data on this question were obtained in the pioneering experiments of Rothschild and Swann (1952), who determined the time of the block to polyspermy in sea urchin eggs. Eggs of Psammechinus miliaris were inseminated with a highly concentrated (> 10^8 spermatozoa per cc) sperm suspension (at 16–18°C). The interaction time was limited by diluting

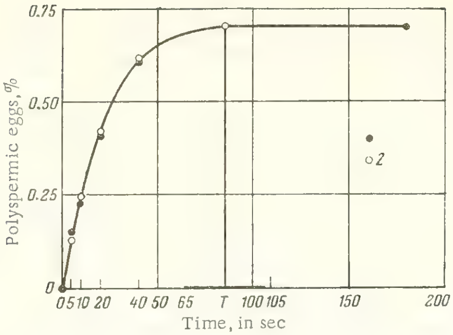

FIGURE 61. Increase in percent of polyspermy in Psammechinus miliaris eggs as a function of duration of gametic interaction (after Rothschild and Swann, 1952).

The abscissa shows the time of interaction of eggs with a concentrated suspension of spermatozoa (9.11×10^7 per cc). 1 — experimental points; 2 — theoretical points. T — time at which percentage of polyspermy does not increase (85 seconds in the experiment shown).

(about 45 times) the suspension with seawater at different times after insemination (reducing the concentration of spermatozoa so that practically no polyspermic fertilization is possible). As the interaction time with the concentrated sperm was increased, the proportion of polyspermic eggs increased to a certain plateau level (Figure 61). The time when the plateau level was reached, indicating that polyspermy was fully blocked, varied from 17 to 94 seconds after insemination in eggs of various females; the average for 14 experiments was 63 seconds.

To estimate to which stage of the cortical reaction this time corresponds, it should be remembered that discharge of the cortical granule contents at 20°C in P. miliaris terminates 40 seconds after insemination (Allen and Griffin, 1958; see Figure 60). At lower temperatures (such as those of the experiments of Rothschild and Swann) this process must last somewhat longer. Correlation of the data indicates that the egg surface becomes completely impermeable to sperm by the time the granule contents are completely discharged or immediately thereafter.

Now to return to what is known concerning the beginning of the blocking process and the appearance of the first changes in the cortex, preventing penetration of supernumerary spermatozoa.

The discharge of the cortical granule is known to be preceded by a long latent period, while a considerable time is also required for the granules to burst over the entire egg surface. The cortical reaction spreads completely only 20—180 seconds after insemination (varying according to temperature and rate of egg development of the various sea urchin species). During this time not a single spermatozoon contacted the egg, and the frequency of polyspermy is generally very insignificant. This fact led Moser (1939) to believe that monospermy is ensured by certain processes occurring in the cortical layer even before the granules burst. This served as a starting point for the hypothesis of a two-phase block to polyspermy elaborated by Rothschild and Swann (1951, 1952; Rothschild, 1956).

According to this hypothesis, certain invisible changes spread in the egg surface in the first 1—2 seconds after gametic contact, rendering it less permeable to other spermatozoa (rapid partial block phase). The second phase is slower and corresponds to changes visible in the cortex; after its completion the egg surface is completely impermeable to spermatozoa (the full block phase).

This hypothesis is a most serious attempt to explain the block to polyspermy as a whole, based upon experimental analysis of the gametic interaction during fertilization (see p. 263). The hypothesis has been widely cited, but not universally accepted; recently serious objections were raised, mainly by the Swedish school of embryologists (Runnström, B. E. Hagström, etc.) who have made a valuable contribution to elaboration of the mechanism blocking polyspermy in echinoderms.

Other views concerning the mechanism protecting the egg from penetration by supernumerary spermatozoa are speculative. Such ideas of Lillie and Tyler based on the fertilizin theory of fertilization have already been discussed (pp. 146–148). Recently Pasteels suggested a single mechanism blocking polyspermy in various animals by molecular changes in the cortex (Pasteels, 1965a, see p. 290), but gave no data concerning the nature of such changes.

6.1. BLOCK TO POLYSPERMY AS A ONE-STEP PROCESS BASED ON THE DISCHARGE OF CONTENTS OF CORTICAL BODIES

6.1.1. Coincidence of Cortical Discharge and Block to Polyspermy

How well does the two-phase block to polyspermy hypothesis actually describe events occurring in the egg as it begins to develop? At which stages of the cortical reaction does the egg surface become less receptive and completely impermeable to spermatozoa? To answer these questions it is necessary to trace step-by-step the change in properties of the egg from the moment of activation until completion of the cortical reaction. In performed such investigations on acipenserids and salmonids (Ginzburg, 1960a, b, 1961a, 1963a, b) and on sea urchins (Ginzburg, 1963c). Unfertilized eggs were activated in some way and inseminated after various time intervals. Changes in the eggs' fertility allowed for ascertaining the time of onset and development of the block to spermatozoan penetration. The capacity of eggs to sense spermatozoa was correlated with the morphology of the cortical layer at the time of insemination.

Acipenserids. Sturgeon eggs were activated by pricking with a glass needle in various regions (Detlaf, 1961a). In the first series, eggs of Acipenser güldenstädti colchicus and Huso huso were pricked near the animal pole (near the micropyle); in the second series pricking was in the region of the vegetal pole (at maximal distance from the micropyle). The eggs were inseminated after various periods of from 1–2 seconds to several minutes (Ginzburg, 1960a, 1961a). If certain invisible changes diminishing permeability spread over the egg surface in the first few seconds after activation, as suggested by Rothschild and Swann, to initiate the rapid partial block phase, then the fertility of eggs in both series would decrease at about the same time. On the other hand, the blocking effect of visibly expressed changes in the cortical layer (secretion of cortical granule material, representing the second phase of the block according to Rothschild and Swann) must occur considerably later in eggs activated near the vegetal pole than in eggs pricked near the animal pole; this accords with the time required for spread of cortical changes from the point where the needle touched to where the spermatozoon penetrates.

The experiments showed that eggs pricked near the animal pole become refractive to fertilization after a very short interval — after 1—5 seconds. Eggs pricked near the vegetal pole remain fertilizable for more than 1—3 minutes (depending on temperature; see Table 33). It is significant that in the second series the same proportion of eggs were fertilized 60 seconds (at 22°C) and 120 seconds (at 15°C) after pricking as in control eggs.

TABLE 33. Experiments of sturgeon eggs pricked in the vegetal region

Interval between pricking and insemination (min)	Temperature, °C		
	13.8	14.5	22.0
1	+	+	+
2	+	+	—
3	+	—	—
4	—	—	—
5	—	—	—

Note. + eggs fertilized; — fertilization impossible.

Parallel to insemination, eggs were fixed and examined cytologically to clarify the condition of the cortical layer near the micropyle at various times after pricking. The patterns obtained were correlated with experimental data on the fertility at the same times.

These results are illustrated by an experiment performed on sturgeon eggs at 13.8°C (Figure 62, Plate XIV, Table 34). In eggs pricked near the animal pole and fixed after 5 seconds, the cortical reaction wave, spreading asymmetrically, reached the boundary of the vegetal hemisphere on the pricked side and embraced the entire animal hemisphere in the opposite direction (Figure 62, a). In microscopic sections of the micropylar locus, discharged cortical granules and cytoplasm are observed (Plate XIV, a). As already mentioned, this pattern is due to the cytological treatment; nevertheless it does indicate the beginning of granule secretion (p. 235).

In this series, the fertility of the eggs was virtually lost within the first few seconds after pricking (Table 34); only an average of 6% of the eggs inseminated after 2 and 5 seconds were fertilized. The rare exceptions of fertilized pricked eggs should be regarded as resulting from individual deviations in duration of the latent period of the cortical reaction (see p. 249).

Not a single pricked egg was fertilized when inseminated after 10 seconds.

In the second series of the experiment (pricking in the region of the vegetal pole) the granule contents still did not reach the marginal zone in eggs fixed after 1 and 2 minutes. (Figure 62, b); the cortical layer of the egg in the micropylar region showed no changes (Plate XIV, b). The percentage of fertilized eggs inseminated after these time intervals was high (87.5% on the average) and was close to the frequency of fertilization in unpricked control eggs in the same batch (84.5%).

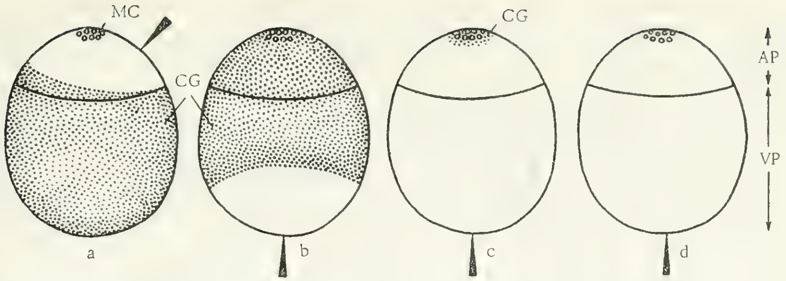

FIGURE 62. Spread of cortical reaction at various times after activation of a pricked sturgeon egg (13.8°C) (schematic):

a — 5 sec after pricking near animal pole; b — 2 min, c — 3 min, d — 4 min after pricking in region of vegetal pole (after Ginzburg, 1960a). AP — animal pole; VP — vegetal pole; CG — cortical granules; MC — micropylar canals.

TABLE 34. Typical results of experiment in which sturgeon eggs were pricked near the animal pole and vegetal pole before insemination (13.8°C)

Interval between pricking and insemination	Pricking near animal pole			Pricking in vegetal pole		
	total eggs	fertilized eggs		total eggs	fertilized eggs	
		number	%		number	%
2 sec	60	2	3.3			
5 sec	40	4	10.0			
10 sec	20	0	0			
1 min				20	16	80.0
2 min				20	19	95.0
3 min				30	13	43.3
4 min				20	0	0
5 min				20	0	0

The cortical reaction encompassed the entire vegetal pole and most of the animal pole by 3 minutes after pricking (Figure 62, c). Vacuolization of the cortical layer around the micropyle was observed in some eggs, but the cytoplasm and membrane remained in contact (Plate XIV, c) whereas they were already separated in other eggs (Plate XIV, d). Accordingly, only 43.3% of the eggs inseminated after 3 became fertilized. After 4 minutes the cortical reaction in these cases completely encompassed the egg surface (Figure 62, d). The cytoplasm was always separated from the membrane around the micropyle, and discharge of the cortical granules was discernible as small tubercles on the inner surface of the membrane (Plate XIV, e). By this time all the eggs had lost their capacity for sensing spermatozoa (Table 34).

These results are typical. In all experiments, whether pricked in the animal or vegetal poles, the cytoplasmic surface became impermeable to spermatozoa at the same time as the cortical granules discharged near the micropyle. In the preceding stage, the pricked eggs were as fertile as control eggs.

Teleosts. Eggs of lake trout Salmo trutta m. lacustris were activated by freshwater and inseminated at various intervals (Ginzburg, 1960b, 1961a, 1963a, b).

It was found that the trout eggs remain fertile for a time (40 seconds at 3.0° and 8.9°C) after immersion in water (see Figure 65). After 60 seconds, fertility decreased sharply and was lost completely within 100–120 seconds. Cytological inspection of eggs from batches fixed at different times after immersion in water showed that fertility decreases when the cortical discharge begins in some eggs in the micropylar region. Total loss of fertility is observed when the cortical alveoli in this region burst in all the eggs.

Exactly as in acipenserids, penetration by spermatozoa in salmonid eggs is blocked at the beginning of the stage of visible cortical changes; until this moment fertility does not decline.

Sea urchins. For comparison in time of development of the cortical reaction and the block to polyspermy, a suitable object is the Murman Coast sea urchin Strongylocentrotus droebachiensis which develops at low temperatures. The same experimental plan as used with fishes was employed; insemination followed activation (Ginzburg, 1963c).

The timing criterion for cortical changes in each batch of eggs was observation of in vivo separation of the membrane. By interrupting the cortical reaction with 4% formalin (see p. 242), it was first found that the membrane starts separating in individual eggs when secretion of the cortical contents already has proceeded in all the eggs (Figure 63). In some eggs, the cortical reaction, which began 30–40 seconds earlier, already encompassed a considerable part of the egg surface. By the time the membrane had completely separated in a portion of the eggs, secretion of the granule contents was completed in all the eggs.

All the experiments were conducted at a constant temperature of 8.0°C ± 0.2 (at which the cortical reaction, including the latent period, lasted about $2\frac{1}{2}$ minutes).

In the first experimental series, the eggs were activated by a 0.05% saponin solution in seawater and then aliquots of eggs were inseminated at various times. Eggs from three females were used. In all cases, fertilization consistently remained at its original high level until membrane separation began in the first eggs (according to in vivo observations) As the cortical reaction developed, the fertility of the eggs gradually declined and was finally lost (Figure 64, a).

In the second series, a spermatozoon was used as the activating agent. Eggs were first inseminated with relatively dilute sperm (10^6 spermatozoa per cc); the second insemination, after different time periods (at 20-second intervals), was carried out with concentrated sperm (10^8 spermatozoa per cc). Experiments on the eggs of 7 females gave comparable results (data on two are given in Figure 64, b). It appears that for a considerable time ($1-1\frac{1}{2}$ minutes) after the initial activating insemination, the second insemination with concentrated sperm gave a high proportion of polyspermy which corresponds to the frequency of polyspermy with only one concentrated batch of sperm (points on the ordinate axis). Thus, the protective changes in the egg surface stimulated by contact between the first spermatozoon and ooplasm, were not observed during this period. A gradual decline in the frequency of polyspermy then occurred, but not to the level characteristic for single insemination (control). Correlation of these data with the results of in vivo observations shows that the block to polyspermy in an egg population first appears synchronously with the beginning of membrane separation in the advanced eggs (Figure 64, b, arrow with letter B). This is the time when, as mentioned before, secretion of the cortical contents begins in all the eggs. By the time separation is completed in the first eggs (Figure 64, b, arrow with letter T) the secretion process has ended in all eggs and polyspermy is definitely blocked.

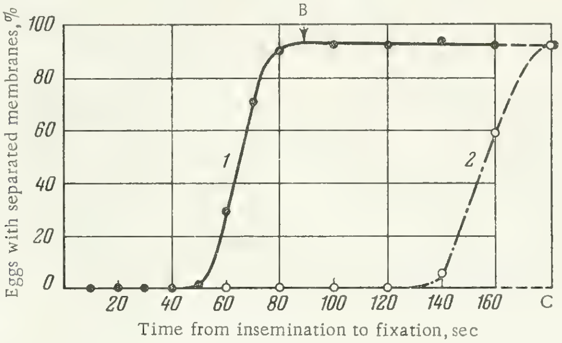

FIGURE 63. Dynamics of the cortical reaction in the sea urchin Strongylocentrotus droebachiensis (after Ginzburg, 1963c).

Concentration of spermatozoa: 1.1×10^7 per cc. Beginning (1) and end (2) of cortical reaction in eggs fixed in 4% formalin at various times after insemination. B — beginning of membrane separation in advanced eggs according to live observations; C — control (percent of eggs with separated membranes, reckoned at the first division stage).

Thus, in all animals studied (acipenserids, salmonids and sea urchins), no signs of changes in the properties of the egg surface were observed in the period preceding discharge of the cortical contents. Such changes (according to two-phase block to polyspermy) would hamper the establishment of effective contact between the gametes.

The block to spermatozoan penetration coincides in all cases in time with discharge of the cortical bodies' contents. It is completed before onset of subsequent changes at the surface of the fertilized egg, such as secretion of hydrophilic colloidal globules in the animal region of the acipenserid oocyte or formation of a hyaline layer in sea urchins.

This coincidence in time is the first evidence that the cortical secretion participates in protecting the egg from penetration by supernumerary spermatozoa. Further evidence is provided by results of experimental inhibition of the cortical reaction and of fertilization of immature eggs.

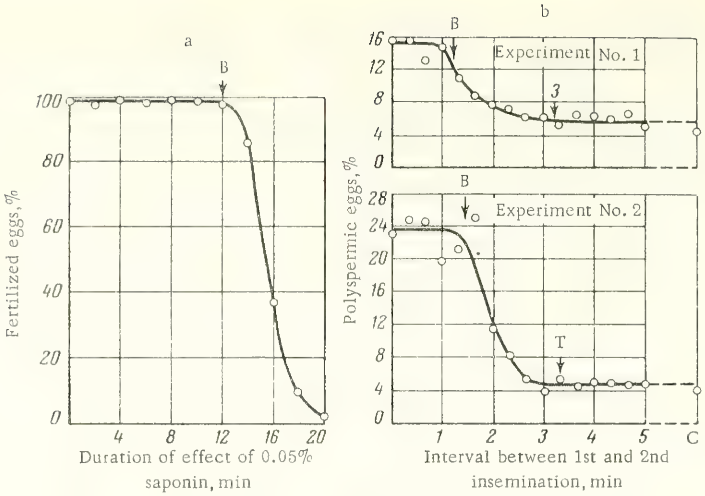

FIGURE 64. Timing of changes in the egg surface preventing penetration of spermatozoa in the ooplasm in experiments on Strongylocentrotus droebachiensis:

a — insemination of eggs activated by 0.05% saponin solution; b — double insemination of eggs with sperms of different concentrations (after Ginzburg, 1963c). Beginning (B) and termination (T) of membrane separation in advanced eggs as observed in vivo. Control (C) with repeated insemination.

6.1.2. Polyspermy as a Result of Inhibition and Total Suppression of the Cortical Reaction

If mature unfertilized sturgeon eggs are treated with urethan, ethyl ether vapor or acetone and then inseminated, the latent period of the cortical reaction is prolonged and secretion of the granule contents is decelerated somewhat. Moreover, the percentage of polyspermic eggs increased several times in all experimental series (Ginzburg, 1960b, 1961a, b).

Inhibition or total suppression of cortical discharge can be obtained in sea urchins using various substances, including narcotics — urethan (Sugiyama, 1956; Aketa, 1961), chloral hydrate (Hagström, 1957; Lönning, 1967b) and nicotine (Hagström and Allen, 1956; Lönning, 1965), inhibitors of oxidative phosphorylation — dinitrophenol and sodium azide (Okazaki, 1956), inhibitors of proteolytic enzymes — ovumucoid, an inhibitor of trypsin from soybeans (Hagström, 1957; Lönning, 1967b), and butyric acid (Aketa, 1961). The same agents disrupt the mechanism blocking polyspermy, and fertilization is polyspermic in a large proportion of cases. The effect of nicotine was already demonstrated by the Hertwigs (Hertwig and Hertwig, 1887) and of urethan by Clark (1936); the effects of the other substances are described in the works cited.

Multiple penetration of spermatozoa occurs in sea urchins under conditions of slow development or absence of the cortical reaction, as well as in fertilization of immature eggs.

The future cortical granules in sea urchin oocytes are dispersed in the endoplasm and occupy a definite position in the cytoplasmic surface layer only when the oocyte is mature. Until the meiotic divisions are completed, spermatozoan penetration of the oocyte does not lead to membrane separation (A. Brachet, 1922). Electron micrographs of such oocytes show that granules distributed in the endoplasm do not undergo changes (Lönning, 1964). Together with this, the block to polyspermy in maturing oocytes is absent or very weak; oocytes of Paracentrotus lividus inseminated at metaphase I or II are penetrated by a number of spermatozoa (on the average from 5 to 10). In the case of insemination at a somewhat later stage (telophase II when the female pronucleus is formed) the number of spermatozoa penetrating decreases (it rarely exceeds 5), but polyspermy is still found in all cases (A. Brachet, 1922).

The experimental data given in this and the preceding section establish without doubt the significance of correlation between secretion of the cortical contents and the block to polyspermy.

Data obtained in Oryzias latipes (Aketa, 1966) seem to contradict such a conclusion. Unfertilized eggs of this fish were treated with 0.2% MS 222 (ethyl m-aminobenzoate methane sulfonate) in isotonic Ringer's solution and were inseminated in it. Although the cortical reaction was totally blocked and began only after the eggs were transferred to the usual medium, fertilization was invariably monospermic. This led Aketa to the conclusion that the block of polyspermy in Oryzias latipes can operate without the cortical reaction. A different explanation of the results seems more likely; in MS 222 solution eggs apparently become fertilized but did not develop, like the salmonid eggs in Ringer's solution or cavity fluid (see p. 159). At this time penetration by supernumerary spermatozoa was impossible, since the fertilizing spermatozoon filled the single micropyle. On transfer to the usual medium the eggs began to develop, the fertilizing spermatozoon was drawn into the cytoplasm and a cortical reaction began and developed the block to polyspermy.

A correlation between the cortical discharge and the block to polyspermy is not sufficient to exclude other processes in protecting the egg from supernumerary spermatozoa. In this connection, the suggested role of the activation impulse in preventing polyspermy shall be discussed.

6.1.3. Activation Impulse and Prevention of Polyspermy

The activation impulse (fertilization impulse or fertilization wave), which is the stimulation wave spreading in the cortical layer of the cytoplasm before visible changes appear, starts a chain of processes in the fertilized egg, which include secretion of the cortical contents, activation of the nucleus quiescent since a certain stage of meiosis, etc. (see T. Yamamoto, 1944a, b; Zotin, 1961a; Dorfman, 1963).

Theorizing from fertilization studied in fishes, Rothschild suggested that polyspermy is prevented in this group by means of the activation impulse (Rothschild, 1958, p. 379). A priori such a view is very unlikely since this impulse can occur both in monospermic eggs, having the mechanism preventing penetration of supernumerary spermatozoa, and in physiologically polyspermic eggs lacking such a mechanism. However, the idea that the activation impulse plays a role in preventing polyspermy can only be refuted by first-hand data.

A single attempt was made in acipenserid eggs to determine the rate of spread of the activation impulse (Detlaf, 1961a, 1962). Eggs of Acipenser güldenstädti and Huso huso were activated by pricking at different distances from the nucleus (in one case at the marginal zone and in another near the vegetal pole). After varying periods, the eggs were fixed and the stage of nuclear division observed in microscopic sections. It was found that the second maturation division is always delayed when there is a larger distance between the point of pricking and the nucleus. The lag was $1\frac{1}{2}-3\frac{1}{2}$ minutes (depending on temperature) and corresponded to the time required for visible cortical changes to spread between the points of pricking in the two series. It follows that the activation impulse in the sturgeon eggs spread at a rate close to that of the wave of cortical discharge.

Thus, discharge of cortical granules at any point on the surface of sturgeon eggs begins almost immediately after the activation impulse reaches this point. This is confirmed by the direct determination of the latent period of the cortical reaction, which is only 1–2 seconds in the animal pole. The lack of any substantial time gap between the activation impulse and the cortical discharge resulting from it makes the sturgeon egg unsuitable for distinguishing between these two processes in the prevention of polyspermy. Such an experiment could easily be done in trout eggs in which the activation impulse and the beginning of the cortical secretion are sharply defined in time (perhaps due to the much lower developmental temperature of these eggs).

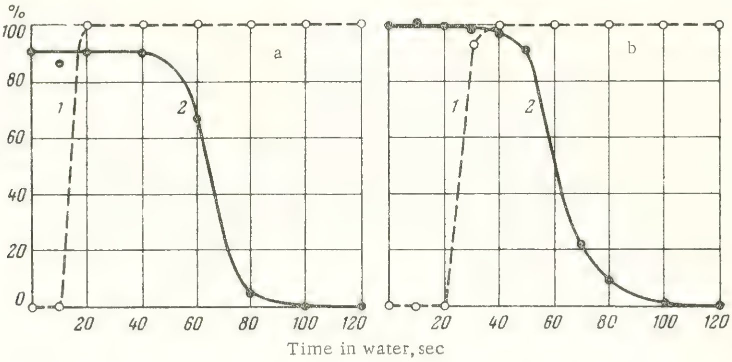

FIGURE 65. Appearance of activation impulse and loss of fertility of lake trout eggs activated in water (after Ginzburg, 1963a). The curves show results of two experiments in eggs of various females conducted at 3.0°C (a) and 8.9°C (b):

1 — percentage of activated eggs in samples transferred to Ringer's solution after activation by water without insemination; 2 — percentage of fertilized eggs in samples inseminated after treatment in water for various times.

The lake trout eggs were activated by freshwater and then transferred to Ringer's solution after different periods of time to determine when the activation impulse started (Ginzburg, 1963a, b). Ringer's solution does not activate salmonid eggs, but allows the activation process, already initiated, to develop

(Kusa, 1950; Devillers et al., 1954; Zotin, 1954). Trout eggs exposed to water for 10 seconds did not show changes in Ringer's solution, but when kept in water for 20–30 seconds (varying in different batches of eggs; see Figure 65, a and b, curve 1) they showed signs of activation after a time in Ringer's solution. A perivitelline space appeared in these eggs, and bipolar differentiation developed (the blastodisc rose above the yolk surface and a fatty zone was formed). This indicates that the activation impulse is stimulated in eggs kept in water for 20–30 seconds. Cytological investigation of such eggs after various times in water indicates that cortical secretion begins only after the eggs were in the water 60 seconds, i. e., 30–40 seconds after the activation impulse appears. It has already been stated that the egg retains its original fertility until the alveolar contents begin to discharge (see p. 258 and Figure 65, curve 2).

Consequently, no changes in the egg surface hampering penetration by the spermatozoa accompany the appearance of the activation impulse.

The meagre data now at our disposal indicate that the activation impulse in fish eggs spreads considerably less rapidly than the hypothesized invisible cortical changes in the first phase of the polyspermy block according to the hypothesis of Rothschild and Swann. As expected, the spread of the activating impulse in the cortical layer is not accompanied by any decrease in the egg's sensitity to spermatozoa.

6.1.4. Does a Phase of Rapid Partial Block to Polyspermy Exist?

The attempts of Rothschild and Swann to substantiate the existence in sea urchin eggs of a first phase of the block to polyspermy will now be briefly described (Rothschild and Swann, 1951, 1952; Rothschild, 1953, 1956). First of all, they determined the collision frequency of spermatozoa with the eggs in suspension, using analogously the system in which spheres are subjected to bombardment by randomly moving gas molecules. Starting from the elementary theory of probability, they next expressed the probability of a successful encounter between the gametes, interpolating the fertilization parameter α which indicates the correlation between the number of gametic encounters per unit time and the frequency of fertilization.

$$\ln \mu = -\alpha t,$$

where μ is the number of unfertilized eggs after t seconds of interaction between the gamtes, and α is the fertilization parameter with the dimension $\sec^{-1}$ characterizing the receptivity of the egg surface to spermatozoa.

Rothschild and Swann then assessed, in terms of the new criteria, the data of experiments involving a fixed time of gametic interaction. They concluded that the parameter α after contact with spermatozoa is only $^{1}/_{20}$ of its value in unfertilized eggs. Thus, the receptivity of the egg surface to spermatozoa decreases by 20 times in the first few seconds after gametic association.

The two-phase block hypothesis was confirmed in the eyes of its authors by the action of nicotine, one of the most effective inducers of polyspermy (Rothschild and Swann, 1950; Rothschild, 1953). Their investigations showed that neither does nicotine increase spermatozoan motility nor does it promote the egg's receptivity to spermatozoa. Thus, the effect of nicotine must be due to a direct influence on the polyspermic-block mechanism. Since nicotine had no effect on dark-field luminescence of the surface layer of the fertilized egg, it was concluded that nicotine does not affect the visible cortical changes or else, it must suppress the invisible changes spreading in egg surface during the hypothetical rapid block phase.

The conclusions of Rothschild and Swann were subsequently disproven, since nicotine, like many other agents inducing polyspermy, inhibits and in certain cases totally suppresses the **visible** cortical changes involved in the process of cortical secretion (Hagström and Allen, 1956; Lönning, 1965). Accordingly, supernumerary spermatozoa penetrate nicotine-treated eggs long after a total block to polyspermy has been established in the control.

The sole remaining argument supporting the existence of a phase of rapid partial block to polyspermy was the change in the parameter α in fertilized eggs.

The attempt to analyze the gametic interaction on the basis of patterns derived from the gas kinetic theory has been repeatedly criticized (Hagström, 1956f; Hagström and Allen, 1956; Hultin, 1956; Runnström et al., 1959; Kille, 1960b). Rothschild and Swann failed to take into account many of the factors which undoubtedly affect gametic association, including the variable quality of eggs and spermatozoa, the varied accessibility of the egg surface to spermatozoa, the chemical interaction between the gametes, particularly the effect of the jelly coat and egg water in inducing the acrosomal reaction even before the spermatozoon contacts the egg surface (causing a sharp decrease in the percentage of spermatozoa capable of fertilizing), etc.

Evidence against the existence of a rapid block phase was provided by experiments with fertilized eggs (Allen, 1954; Allen and Hagström, 1955; Hagström and Runnström, 1959) in which the cortical reaction was interrupted by heating 5—20 seconds after contact with spermatozoa, i. e., after the hypothetical rapid block phase .When such eggs were inseminated a second time, the spermatozoa randomly penetrated the part of the surface where cortical granules remained intact (the frequency of polyspermy in each experimental series was virtually equal to the percent frequency of fertilized eggs). This indicates the absence of any stable block to polyspermy during the first few seconds after fertilization.

Direct testing of the receptivity of the egg surface to spermatozoa conducted in fish and sea urchin eggs, which has been discussed thoroughly (Ginzburg, 1960a, b; 1961a; 1963a, b, c; p. 255 ff.), could show certain changes in receptivity if such a first phase rapid block existed even if it is reversible. However, the eggs remained accessible to spermatozoa in all cases, both in the first few seconds after activation and thoughout the latent period of the cortical reaction.

At present, no experimental data confirm the existence of a first phase of rapid block to polyspermy. On the contrary, results of various experiments indicate that there are no changes in the egg surface hampering contact between the gametes until the cortical secretion begins.

6.1.5. Effect on Spermatozoa of Substances Secreted from the Egg during the Cortical Reaction

The close correlation between the cortical secretion and the block to polyspermy led to the assumption that the blocking mechanism involves the direct effect of cortical substances on spermatozoa. This assumption can be proved experimentally by reproducing processes occurring on the surface of the fertilized egg in vivo, by mixing a drop of sperm with perivitelline fluid containing discharged substances from the egg.

Such experiments were performed on lake trout eggs (Ginzburg, 1960a, 1961a), usually several hours after fertilization or activation when the perivitelline space had already reached a considerable size. The trout eggs were dried on filter paper, the membrane was pricked with a needle above the animal pole and the perivitelline fluid collected with a pipette. When the fluid was mixed with a drop of sperm, intensive agglutination of the spermatozoa was observed (see Figure 50). The tails became twisted and stuck together in fantastic loops. Both heads and tails of spermatozoa adhered. The agglutinating effect was achieved not only in pure perivitelline fluid, but also when diluted 4—8 times in water. The agglutinins in the perivitelline fluid are quite stable, and were still found after 12—13 days of incubation.

Substances secreted by the fertilized sea urchin egg also can agglutinate spermatozoa, as demonstrated by Motomura (1953) in experiments on Strongylocentrotus nudus, in which jelly coat and vitelline membranes of mature unfertilized eggs were removed and the eggs treated in a solution of NaSCN. The naked unfertilized eggs did not release agglutinins into the water. The discharge began immediately after fertilization, and water containing the eggs possessed a strong agglutinating effect 3 minutes after insemination. Secretion of agglutinins continued for 10 minutes after insemination, and then ceased. Discharged substances retained agglutinating properties for a long time; even after the embryo leaves its membranes, the water has a high titer of agglutinins.

Egg secretions, preventing penetration of supernumerary spermatozoa into the cytoplasm never cause their agglutination. In mammalian eggs, spermatozoa which have penetrated the zona pellucida of the fertilized egg, swim freely in the perivitelline fluid (Austin and Braden, 1956). Apparently, the substances discharged by the egg in this case change the surface of the sperm head (or, more probably, the egg membrane itself) so as to render gametic association impossible.

A model for such changes is the loss of spermatozoan fertility by treatment with a synthetic polymer inhibiting hyaluronidase and other enzymes (Parkes et al., 1954). Rabbit spermatozoa treated with this preparation remain motile and could penetrate the egg membrane, but had a sharply reduced capacity to establish effective contact with the egg cortical layer. Unfertilized eggs with spermatozoa lying freely in the perivitelline space were repeatly encountered, which is very rare in normal conditions of insemination.

If supernumerary spermatozoa are blocked from penetrating directly by egg secretions, the removal of these substances from the egg surface should render the ooplasm permeable.

Experimental data examined in the following section show that the block to polyspermy actually is reversible.

6.1.6. Reversibility of the Block to Polyspermy on Removal of Secretions from the Egg Surface

It is widely believed that the changes in the egg surface on fertilization or artificial activation are not reversible and that spermatozoa can penetrate a developing egg only while the cortical reaction remains uncompleted (Lillie, 1919, pp. 161 ff.; A. Brachet, 1931; Just, 1939).

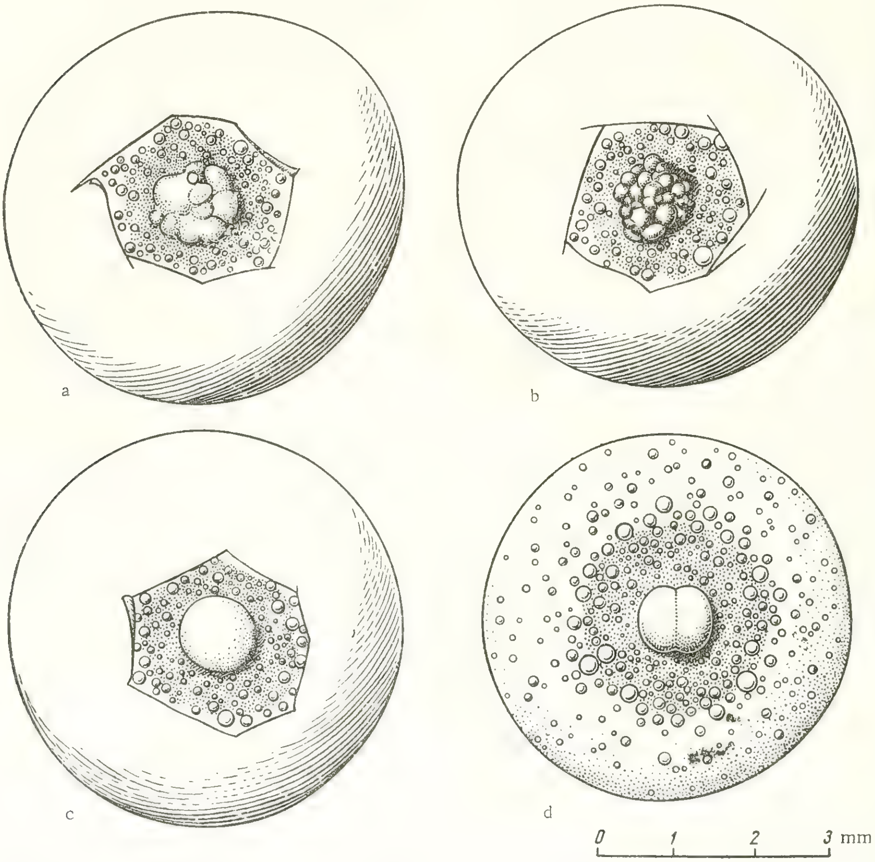

FIGURE 66. Reversibility of the block to polyspermy in lake trout eggs after removal of the perivitelline fluid.

The structure of blastodiscs was followed 18 hours after insemination (at 6.3–6.8°C); in eggs activated by water, inseminated after 120 min through the aperture in membrane (a, b, c), after washing out of perivitelline fluid (a, b) and without washing out (c). The intact control egg (d) taken from cavity fluid was inseminated together with activation (membrane removed after fixation).

However, this view is erroneous, since it was based on experiments of insemination of developing eggs whose outer covers remained intact. Actually, the surface of the activated egg itself is permeable to sperm, whose penetration is blocked by membranes whose properties were changed by substances discharged from the egg, by the perivitelline fluid and by other extracellular protective formations. These conclusions were derived from experiments, described below, aimed at studying the mechanism protecting trout eggs from penetration by supernumerary spermatozoa. A number of other investigations on fishes and sea urchins, performed for different aims, led to similar results since their data could be used for analysis of the mechanism blocking polyspermy.

Experiments on fishes. A small aperture was cut in the membrane of lake trout eggs previously fertilized or activated by water. Such eggs were inseminated with a dense sperm suspension. The spermatozoa agglutinated on contact with the perivitelline fluid; subsequent changes in such eggs did not differ from the development of control eggs with intact membranes. When the perivitelline fluid was washed thoroughly out of the membrane and a drop of sperm introduced through the aperture, several or even many spermatozoa regularly penetrated the cytoplasm of the developing eggs. The heads of these spermatozoa were transformed into pronuclei, which divided and formed a large number of small blastomeres (Ginzburg, 1960b, 1961a, unpublished data; see Figure 66).

The fertilization rate in such eggs was practically the same with insemination at different periods (from 25 to 120 minutes) after activation (Table 35). Penetration by spermatozoa was observed even 24 hours after activation in water.

TABLE 35. Fertilization of activated trout eggs (with aperture in membrane)

Method of insemination	Period from activation in water to insemination, min	Total eggs	Fertilized eggs	
			number	%
Perivitelline fluid washed out and sperm introduced under the membrane	25–45	33	23	69.7
	60	59	32	54.2
	90	36	24	66.7
	120	37	21	56.8
Control (sperm placed in water)	20–45	16	0	0
	60,120	18	0	0

These results led to the conclusion that in fertilized or activated eggs the plasmallema remains permeable to sperm. This conclusion is supported by data on multiple spermatozoan penetration in developing eggs of other fish species whose membranes were completely removed.

By cutting away part of the membrane, the Clupea harengus pallasi egg can be freed (Yanagimachi, 1957d). When these membraneless eggs, either unfertilized or prefertilized or activated, were inseminated, multiple spermatozoan penetration and irregular cleavage (characteristic for polyspermy eggs) were observed.

The membranes of water-activated eggs of Misgurnus anguillicaudatus and Acheilognathus lanceolata, as well as fertilized Misgurnus eggs, were removed with 1% trypsin solution (Gamo et al., 1960). When inseminated, such naked eggs were penetrated by several spermatozoa, numerous male pronuclei and seminal asters were formed and cleavage was atypical with formation of a large number of small blastomeres. The polyspermic embryos died generally at the blastula-gastrula stages.

In these experiments, substances discharged from the cortical alveoli were not retained at the egg surface due to removal of the membranes, and the surface remained unprotected from multiple spermatozoan penetration.

The developing egg's surface is permeable to spermatozoa, not only after products of the cortical reaction are removed, but also when the cortical secretion is completely blocked.

If unfertilized chum salmon eggs are subjected first to acidified Ringer's solution and then 0.2% pancreatin for removal of the membrane, they become activated (the blastodisc is sharply delineated, the second maturation division is completed and the female pronucleus is formed), but the cortical alveoli do not burst. When inseminated, such activated naked eggs begin to cleave, retaining the unchanged alveoli in the cortical layer (while activated uninseminated eggs in the control do not cleave). Cleavage is characteristic for polyspermic fertilization (Kanoh and Yamamoto, 1957).

Experiments on sea urchins. As in fishes, the surface of the fertilized or activated sea urchin egg is permeable to spermatozoa after elimination of all products of the cortical reaction — the fertilization membrane (including cortical granule material), perivitelline fluid and hyaline layer.

If the fertilization membrane is removed mechanically or enzymatically and the hyaline layer dissolved and the eggs placed in a medium lacking bivalent cations (Ca and Mg ions) or in a urea solution (1 M), spermatozoa can penetrate even up to the 2- and 4-blastomere stages (Ishida and Nakano, 1950; Sugiyama, 1951; Hagström and Hagström, 1954b; Nakano, 1956b; Tyler et al., 1956).

Experiments on 5 sea urchin species (Hagström and Hagström, 1954b) indicate that the block to polyspermy is removed in direct proportion to the degree of dissolution of the hyaline layer. The percentage of polyspermic eggs after the second insemination is small if the hyaline layer swells and disintegrates only slightly but if totally lysed, intensive polyspermy is observed.

Simple removal of the fertilization membrane, without dissolution of the hyaline layer, was insufficient to allow penetration of spermatozoa into most sea urchin eggs. In some species (Lytechinus pictus and L. variegatus; Tyler et al., 1956), however, a high percent (up to 100%) of polyspermy results from the second insemination of the fertilized eggs whose fertilization membrane was removed mechanically.

Naked developing sea urchin eggs often showed a high intensity of polyspermy even when inseminated with a moderately concentrated suspension of about 10^6 spermatozoa per cc; many seminal asters were visible in the ooplasm and the eggs cannot cleave, indicating penetration by many (5 or more) spermatozoa. This gives us grounds to believe that eggs, having undergone the cortical reaction and then liberated from discharged substances, completely lose the apparatus protecting them from multiple spermatozoan penetration.

Other animals. The reversibility of the block to polyspermy after cortical reaction products are eliminated was also shown in an enteropneust, Saccoglossus kowalevskii (Colwin and Colwin, 1957b). If membranes and other layers surrounding fertilized or activated eggs are removed, the cytoplasmic surface again becomes permeable; a second fertilization is possible right up to the early blastula stages. Electron micrographs (Colwin and Colwin, 1967) show that spermatozoa penetrate blastomeres in the cleaving egg exactly as they do an unfertilized egg.

6.1.7. Conclusion

Exhaustive investigations into the mechanism blocking polyspermy carried out on fishes and sea urchins indicate that it is a one-step process based on discharge of the contents of cortical bodies (granules or alveoli). This conclusion rests on a combination of all the evidence given above that the block to polyspermy coincides in time with secretion of the cortical contents, inhibition of this process is consistently accompanied by multiple spermatozoan penetration, the secretions agglutinate spermatozoa in vitro and the egg surface is rendered permeable to spermatozoa after removal of the discharged substances.

There is no experimental proof of the participation of any earlier cortical changes in the block to polyspermy, such as the activation impulse or other invisible changes postulated in the two-phase block to polyspermy.

There are also no grounds for attributing any role to processes occurring after completion of the cortical secretion, such as formation of the hyaline layer in sea urchins or the discharge of hydrophilic colloidal globules in sturgeons.

Having clarified the general basis of the block to polyspermy, the function of the mechanism protecting against multiple penetration of spermatozoa in different animals will now be examined, taking into account the egg structure (particularly, egg membranes) and conditions of gametic association.

6.2. BLOCK TO POLYSPERMY IN DIFFERENT ANIMALS

6.2.1. Fishes

Acipenserid and teleost eggs are covered by dense sperm-impermeable membranes over their entire surface, except for the micropylar canals. Thus, the mechanism blocking polyspermy in fishes is very simple and efficient.

Teleost egg membranes have a single micropyle. The fertilizing spermatozoon, having passed through the micropylar canal and contacting the cytoplasm, stimulates the activation impulse and the chain of processes terminating in discharge of alveolar contents. The terminal part of the micropylar canal is just wide enough to accomodate a single spermatozoon (see Table 14 and Figure 49), so that subsequent spermatozoa cannot come in contact with the ooplasmic surface when the fertilizing spermatozoon is located at the end of the micropyle. A little later, when the spermatozoon is already inside the egg, cortical alveolar material is secreted in the micropylar region. These substances cause changes in the spermatozoan cell membrane leading to their agglutination. This effect alone is sufficient to prevent spermatozoa from passing through the perivitelline space and approaching the ooplasmic surface.

The mechanism blocking polyspermy in teleosts is dependable under any insemination conditions; fertilization is invariably monospermic no matter how high the spermatozoan concentration around the egg and whatever the physiological state of the eggs. Various experimental agents (urethan solution, chloroform vapor, ethyl ether, acetone) inducing polyspermic fertilization in other animals prove ineffective when applied to lake trout (Ginzburg, unpublished).

The acipenserid egg membranes have several, sometimes many micropylar canals, so that there is a real possibility that several spermatozoa can penetrate the egg. However, the micropyles are concentrated in a small section at the animal pole and the cortical reaction affects this entire region within a very short time. The rapid blocking of all the micropyles after a single spermatozoon has penetrated the ooplasm inside one of them is not only ensured by their proximity but also by the short latent period of the cortical reaction (usually lasting only 1–2 seconds and much shorter than in teleosts). Several spermatozoa penetrate the egg only when they simultaneously touch the cytoplasm in different micropyles or if the cortical reaction spreads slowly.

Experimental polyspermic fertilization can be obtained in sturgeons by inseminating with a highly concentrated spermatozoan suspension and by the action of inhibitors of the cortical reaction. Even in such cases, the number of polyspermic eggs rarely exceeds 30–40% (whereas under similar conditions the frequency of polyspermy in sea urchin eggs may reach 100%).

A comparatively high percentage of polyspermy in sturgeons is sometimes encountered in fish hatcheries, where it is due to the low quality of the eggs or defective biotechnics of the artificial insemination.

In nature, fertilization of sturgeon eggs apparently may be polyspermic only exceptionally, since the female spawns mature eggs in optimal physiological condition when external conditions are favorable and the spermatozoan concentration is not high since sperm is discharged in rapid currents which immediately disperse it.

6.2.2. Sea Urchins

The block to polyspermy in sea urchins, as in fishes, is mediated by discharge of the cortical contents from the egg. Unlike fishes, the membranes of sea urchin eggs are permeable to sperm over the entire surface and remain so during the latent period of the cortical reaction; during the active phase of the cortical reaction a progressively decreasing part of the egg surface remains permeable. How, then, is multiple penetration by spermatozoa prevented under such conditions?

Rothschild and Swann ingeniously attempted to explain the contradiction between the permeability to sperm and monospermic fertilization by a two-phase block to polyspermy; however, their hypothesis was disproven experimentally (see pp. 263–264).

The discussion of the function of the mechanism preventing polyspermy in sea urchins should convince the reader than this contradiction is imagined rather than real.

First of all, the jelly coat plays a considerable role in preventing polyspermy in sea urchins, as conclusively shown in Hagström's series of investigations. If the jelly coat is removed very carefully with acidified seawater at a pH about 5.8 (Hagström, 1956a, b) or mechanically (Hagström and Markman, 1957), the time required for effective gametic contact is reduced. Removal of the jelly coat also leads to a considerable decrease in the spermatozoan concentration required for 100% fertilization (Hagström, 1956b). Comparison of rates of the gametic association under various conditions shows that about 80–90% of the spermatozoa passing through the jelly coat lose their fertilizing capacity (Hagström, 1959b). Jelly-coat substance diluted in seawater affects spermatozoa much like the membrane in situ (Hagström, 1959a). The cause of this phenomenon may be due to the capacity of jelly coat substance to induce an acrosomal reaction in spermatozoa (see p. 167), which renders them infertile if they are not in contact with the egg surface.

An important role in the prevention of polyspermy in sea urchins has been also attributed at various times to the other protective egg membranes. Fol (1877a, b, c, 1877–1879), who recognized the primary dependence between the cortical reaction and the block to polyspermy, suggested that the active mechanism is the fertilization membrane (which was the sole effect of the cortical reaction observable at that time). Hagström (Hagström and Hagström, 1954b; Allen and Hagström, 1955; Hagström, 1956e, 1957) considers the hyaline layer of the fertilized egg to be highly significant in this connection. However, since the block to polyspermy appears along with secretion of cortical contents and is complete before either the fertilization or hyaline layers begin to form, these structures evidently can play no part in protecting the egg from penetration by supernumerary spermatozoa during fertilization.

Moreover, it is certain that the fertilization membrane has a protective function at the later stages of embryonic development, after completion of the process of the block to polyspermy, as a mechanical obstacle preventing spermatozoa from approaching the egg surface and isolating the perivitelline fluid and hyaline layer from the surrounding medium (its experimental removal was sufficient for secondary fertilization to take place). As correctly noted by Rothschild (1958), the hyaline layer could protect the intact egg only if the spermatozoa were capable of overcoming the external fertilization membrane, which actually does not happen.

Among the protective formation surrounding the sea urchin egg, only the jelly coat plays a significant role in preventing polyspermy. The action of the jelly-coat material eliminates most of the spermatozoa. However, spermatozoa contacting the egg surface without becoming infertile and while it is still unprotected by a cortical discharge should be able to penetrate the cytoplasm. Why then is the percentage of polyspermic eggs usually so very low?

A solution to this question is provided in an analysis of the experimental data of B. E. Hagström et al. (Table 36) on the kinetics of fertilization in an egg population, obtained by restricting the interaction time of the gametes. In addition to the values for 12 batches of eggs (Hultin and Hagström, 1956), additional data on the control curves of various insemination experiments were taken from other works indicated in the table. In these controls, the eggs, whose jelly coats were intact, were inseminated with moderately concentrated suspensions of 5×10^5 to 2.6×10^6 spermatozoa per cc. At various times after insemination (5, 10, 20 seconds or more), unattached spermatozoa in the samples were inactivated by sodium lauryl sulfate. All the data used refer to good-quality eggs giving close to 100% fertilization.

It is known that the probability of a complex event, consisting of the coincidence of two simple events, is equal to the product of the probabilities of these two events if they are independent of one another. The establishment of effective contact between the egg and two different spermatozoa represents such independent events. Accordingly, the probability on dispermic fertilization will be equal to the square of the frequency of monospermic association determined experimentally as a single value (since it may be assumed that the chances of two different spermatozoa associating with the egg are, on average, the same). The estimated probabilities of dispermic fertilization within 5–20 seconds after insemination for different batches of eggs are given in Table 36.

Unfortunately, the percentage of polyspermy was not determined together with the percent fertilization in experimental material included in this table. The following typical results are known only for Paracentrotus lividus: there is 1% polyspermy with a suspension containing 2.5×10^6 spermatozoa per cc, and 8% with 1.3×10^7 spermatozoa per cc (Hagström, 1956b). However, it should be remembered that eggs of different females show a greatly varying tendency to polyspermy. Thus, the percent of polyspermy in 5 batches of Strongylocentrotus droebachiensis eggs inseminated with suspensions of $2.38-4.20 \times 10^6$ spermatozoa per cc varied from 0.8 to 10.7% (Ginzburg, 1963).

Data for the two sea urchin species, given in Table 36, indicate that the probability of dispermy either 5 or 10 seconds after insemination does not exceed the actual percent of polyspermy in the vast majority of cases of eggs inseminated with the same sperm concentration. This is particularly evident in the case of Paracentrotus lividus if the mean values are calculated excluding the three deviating batches (nos. 10, 11, and 13). The probability of dispermic fertilization 10 seconds after insemination is then 1%.

The unusually high fertilization rate of these three deviating batches might be related to the great ripeness of the eggs or the frequent loss of the jelly coat (which was observed in eggs of several females of S. droebachiensis). Unfortunately, it is not known whether the high probability of dispermic fertilization in these batches was actually expressed in a high percentage of polyspermy. The frequency of polyspermy may in these cases not exceed the usual limits, if the cortical reaction begins earlier in the eggs

TABLE 36. Probability of dispermic fertilization in sea urchin eggs within 5–20 seconds after insemination

No.	Species	Number of spermatozoa per cc	Fertilized eggs at various times (in sec) after insemination (in %)			Probability of dispermic fertilization at various times (in sec) after insemination (in%)			Author
			5	10	20	5	10	20	
1	Paracentrotus lividus	5×10^5	1	3	24	0.01	0.09	5.76	Hagström and Hagström, 1959, Figure 2
2		1×10^6	1	7	25	0.01	0.21	6.25	Ibid, Figure 3
3		1.5×10^6	0	4	10	0	0.16	1.0	Hagström, 1956a, Figure 1
4		2×10^6	0.58	7.04	45.67	0.003	0.50	20.88	Hultin and Hagström, 1956, experiment I ♀ 1
5		2×10^6	1.37	20.51	72.90	0.02	4.20	53.14	Ibid, experiment I ♀ 2
6		2×10^6	0.34	2.08	22.07	0.001	0.04	4.88	Ibid, experiment I ♀ 3
7		2×10^6	1.31	8.85	28.14	0.02	0.79	7.90	Ibid, experiment II ♀ 1
8		2×10^6	0	1.06	9.25	0	0.01	0.86	Ibid, experiment II ♀ 3
9		2×10^6	0.81	2.35	3.26	0.006	0.06	0.11	Ibid, experiment II ♀ 3
10		2×10^6	16.86	54.31	77.78	2.86	29.48	60.37	Ibid, experiment III ♀ 1
11		2×10^6	9.56	70.76	98.63	9.22	50.13	97.22	Ibid, experiment III ♀ 2
12		2×10^6	0.58	6.05	25.98	0.004	0.37	6.76	Ibid, experiment III ♀ 3
13		2×10^6	8.51	63.41	89.88	0.72	40.20	80.82	Ibid, experiment IV ♀ 1
14		2×10^6	0	1.38	17.59	0	0.02	3.10	Ibid, experiment IV ♀ 2
15		2×10^6	4.21	30.57	94.47	0.18	9.36	89.30	Ibid, experiment IV ♀ 3
16		2.6×10^6	0	3	7	0	0.09	0.21	Hagström and Hagström, 1954a, Figure 1
		Average for all batches				0.82	8.48	27.41	
		Average, excluding nos. 10, 11, and 13				0.02	1.22	15.40	
17	Psammechinus microtuberculatus	1×10^6	—	7	22	—	0.49	4.84	Hagström, 1957, Figure 1
18		1×10^6	—	3	14	—	0.09	1.96	Hagström, 1956c, Figure 1
19			—	8	25	—	0.64	6.25	Hagström and Markman, 1957, Figure 2
			Average			—	0.41	4.35	

which react more rapidly with spermatozoa (the great variations in the length of the latent period of the cortical reaction has already been mentioned, see pp. 250–251).

Having demonstrated that the calculated probability of dispermic fertilization 10 seconds after insemination is close to the experimentally-found frequency of polyspermy, we can conclude that supernumerary spermatozoa associating with the egg in the first 10 seconds actually penetrate the cytoplasm. Only those spermatozoa which become attached later, i. e., immediately before the cortical secretion begins or while the secretion process spreads over the egg surface, are eliminated.

Let us examine the possible mechanism of such elimination.

Electron micrographs of eggs of three sea urchin species $2-3\frac{1}{2}$ minutes after insemination (Afzelius and Murray, 1957) showed that all the spermatozoa in contact with the vitelline membrane had undergone at least a partial acrosomal reaction; in most cases the reaction was complete and the end of the acrosomal filament was in contact with the cortical cytoplasm. Obviously, not all these spermatozoa can penetrate the egg. Those spermatozoa contacting the cytoplasm late are separated during the cortical reaction exactly like a single fertilizing spermatozoon in an egg killed by 4% formalin before the cortical reaction has spread over a considerable part of the surface. The fertilizing spermatozoon, not yet sufficiently embedded in the cytoplasm, is torn out of the egg by the separating membrane on swelling of the discharged cortical contents (Allen and Griffin, 1958; see Figure 59).

An attached spermatozoon may also not be able to penetrate if it is attached in an unfavorable place. Although sea urchin spermatozoa can penetrate at any point on the egg surface, it has been shown that penetration occurs most frequently in a certain region at the level of the marginal zone and somewhat below it (Runnström, 1961, 1962). This author suggested that the cortical cytoplasm in this region reacts more rapidly to the spermatozoan acrosome than in the rest of the egg surface.

On the whole, the mechanism blocking polyspermy in the sea urchin egg can be depicted as follows. During the latent period of the cortical reaction the egg surface is liable to penetration by spermatozoa; the unprotected surface gradually decreases only when cortical changes begin. Nevertheless, the intensity of polyspermy is generally very slight, due to two reasons.

1. The penetration of the ooplasm by spermatozoa coming in contact with the egg surface during the latent period is unimpeded. When natural conditions of insemination are simulated (eggs retain the jelly coat and the concentration of spermatozoa in suspension is moderate), the probability of several spermatozoa establishing such contact simultaneously or within the latent period is very low and corresponds to the frequency of polyspermy actually observed.

2. Spermatozoa attaching to the egg late during the spread of the cortical reaction (or which have attached in a weakly reactive region) are torn away as the membrane separates.

Thus the data at our disposal indicate that the mechanism blocking polyspermy in sea urchins, as in fishes, is based on the secretion of the contents of cortical bodies. This process leads to the formation of a barrier protecting the egg surface from spermatozoa and to the tearing away of spermatozoa not yet taken into the cytoplasm.

This second aspect of the blocking function of the cortical reaction — tearing away of supernumerary spermatozoa — was not dealt with up to now, since it hardly played a role in fishes with their specific limitation of the sperm-permeable surface and conditions of direct contact between the sperm head and ooplasm. In sea urchins having a sperm-permeable membrane all over the surface, tearing away of supernumerary spermatozoa during the cortical reaction apparently is essential in blocking polyspermy.

6.2.3. Other Animals

The correlation between the presence of cortical bodies (granule or alveolus) and monospermic fertilization was demonstrated earlier in eggs of animals of 6 different phyla. This naturally leads to the assumption that the block to polyspermy based on the cortical secretion is widespread, and not confined to fishes and sea urchins. Unfortunately, investigations into the mechanism blocking polyspermy in other taxonomic groups have not been carried out, and the discussion will necessarily be restricted to isolated observations and certain views on the function of this mechanism.

Annelids. From data in the previous chapter, it is clear that the cortical reaction in polychaetes proceeds in very diverse ways. It is basically similar in nereids to the cortical changes in sea urchin eggs; first-hand observations of fertilization in Nereis limbata (Lillie, 1911) show that the products of the cortical reaction, which infiltrate through the vitelline membrane, remove all spermatozoa attached to the egg surface except for a single fertilizing spermatozoon. The mechanism blocking polyspermy in this case is very likely similar to that found in sea urchins.

The initial phases of the cortical reaction in Sabellaria and Pomatoceros often occur before gametic contact is established (see p. 244). This results in a significant decrease in the sperm-permeable egg surface, apparently limiting it to the points of contact between the cytoplasmic strands and the vitelline membrane. This too may act as an additional factor in eliminating spermatozoa.

Additional research is required to clarify the mechanism blocking polyspermy in Chaetopterus and the echiuroids.

Bivalve mollusks. Investigations of the cortical reaction in representatives of this group showed (see p. 245) that morphological changes in the cortex during fertilization are usually very inconspicuous, but even in these cases observations indicate that certain substances are secreted by the fertilized egg.

If the cortical reaction in Ostrea gigas eggs is inhibited by nicotine and urethan (which delay the cortical discharge in sea urchins and fishes and are standard inducers of polyspermy in them), the frequency and intensity of polyspermy increase sharply (Ginzburg, unpublished). This indicates the basic similarity between the mechanism blocking polyspermy in bivalve mollusks and other physiologically monospermic animals.

The motility rate of spermatozoa of bivalve mollusks is relatively low (it is approximately 3 times higher in sea urchins and salmonids than in Ostrea gigas, see Table 23) and the gametic association takes unusually long (the time required for a maximal fertilization percentage to be reached in Ostrea gigas is twice that in acipenserids and salmonids, see p. 177). These features probably compensate somewhat for the weak reaction of the egg to attachment of the fertilizing spermatozoon. Nevertheless, the mechanism blocking polyspermy in this group is incomparably less effective than in sea urchins; when eggs of Barnea spinosa (Pasteels, 1965a) and Ostrea gigas (Ginzburg, unpublished) are artificially inseminated, some are always polyspermic in spite of dilution of the sperm.

The electron microscope observations of fertilization in B. spinosa showing no reaction of the egg to association with a spermatozoon led Pasteels to the conclusion that the block to polyspermy in Bivalvia takes place at the molecular level and is unrelated to morphologically expressed cortical changes (Pasteels, 1961, 1965a). It seems, however, that the cortical changes in bivalve mollusk eggs have been studied far too little to exclude participation in the block to polyspermy of substances, albeit in small amounts, discharged by the fertilized egg.

Echinoderms (except for sea urchins). The mechanism blocking polyspermy has not been studied in starfishes, whose cortical reaction closely resembles that in sea urchins. Study of fertilization in Astropecten auranciacus (Hörstadius, 1939) showed that the membrane begins to separate after the fertilizing spermatozoon is trapped in the fertilization cone, 4–5 minutes after it becomes attached to the jelly coat. Supernumerary spermatozoa which have undergone acrosomal reactions, (since fertilization cones protrude toward them from the egg surface) are detached from the egg together with the membrane. These observations of Hörstadius confirm my belief, based on data on fertilization in the sea urchin, that the supernumerary spermatozoa, which attach to the egg late, begin penetrating the ooplasm but fail to complete this before the perivitelline space is formed, and are detached along with the separating membrane.

Cyclostomi. Careful first-hand observations of fertilization in Lampetra fluviatilis and L. planeri (Kille, 1960a) indicate that the cortical reaction protects the egg from penetration by supernumerary spermatozoa in Cyclostomi.

Two spermatozoa penetrate the egg only when these approach and attach to the membrane almost simultaneously. If a second spermatozoon approaches the egg when the cortical alveoli have already begun discharging, it not only does not penetrate the cytoplasm but it cannot even enter the outer layer of the membrane (which the first spermatozoon does without impediment).

Monospermic fertilization in the lamprey is undoubtedly ensured mainly by the limitation of the area permeable to sperm; if the total egg surface of the European lamprey is 4.67 mm^2, the area of the jelly excrescence through which alone spermatozoa can penetrate the cytoplasm, is only 0.04 mm^2 (Kille, 1961). This limitation makes it very unlikely that more than one spermatozoon attaches before the cortical reaction begins.

Urodele amphibia. Bataillon demonstrated the importance of the cortical reaction in protecting the frog egg from penetration by supernumerary spermatozoa. If the cortical reaction is retarded, fertilization is regularly polyspermic; these results were obtained when a Rana temporaria egg was heated before fertilization (Bataillon, 1909) and when frog eggs were inseminated and subsequently kept in a 25% NaCl solution or isotonic (0.043 N) solutions of NaI and NaBr (Bataillon, 1919; see also A. Brachet, 1931, p. 100).

Mammals. Since it has already been determined that in some mammalian eggs there are cortical granules whose contents are discharged on fertilization, there are grounds for believing that the block to polyspermy in mammals has the same basis as in sea urchins, fishes and a number of other animals.

During a study of gametic association patterns in mammals, particular attention was drawn to egg membrane changes occurring on fertilization which lead to the elimination of spermatozoa around the egg, and thus contribute to the prevention of polyspermy.

These changes or reactions of the zona pellucida (and called "zona reaction") lead to very diverse results in different species (Braden et al., 1954; see Austin, 1961). In some species (hamster, vole, dog, sheep) they are so effective that none of the spermatozoa succeed in penetrating the perivitelline space of the fertilized egg. In others (rat, mouse, guinea pig, cat, polecat) these changes only reduce the number of spermatozoa passing through the zona pellucida, so that in a considerable proportion of cases an additional spermatozoon (or, occasionally, several) is found in the perivitelline space. Finally, in certain species (rabbit, pika, mole), the membrane changes must be only slight, since many additional spermatozoa (reaching 200—300 in the rabbit) regularly accumulate in the perivitelline space.

The changes in the zona pellucida, which make it difficult or impossible for spermatozoa to penetrate it, apparently result from discharge of certain substances during the cortical reaction, similar to the transformation of the vitelline membrane accompanying cortical secretion in the sea urchin egg (Austin, 1961).

Indirect proof of this view is provided by the wavelike nature of the spread of the changes in the zona pellucida. The sperm traces were determined in the zona pellucida of rat eggs, through which two spermatozoa had passed; it was found that spermatozoa penetrated considerably more frequently in the hemispheres of the egg than in a single hemisphere (Braden et al., 1954). It is therefore probable that the reaction of the zona pellucida (as well as the process underlying it occurring at the cortical level) spreads from the first point of contact of the spermatozoon.

The same is indicated by deceleration of the reaction of the zona pellucida by aging and heating, which inhibit the cortical reaction in the sea urchin egg (see Austin and Bishop, 1957a).

6.2.4. Common and Diverse Features of the Mechanisms Blocking Polyspermy

Common basis of the block to polyspermy. All the data examined indicate that the eggs of various remotely related groups of animals are protected against penetration by supernumerary spermatozoa by discharge of the contents of cortical bodies.

This directly contradicts the conclusions of Pasteels in his reviews devoted to the cortical reaction (Pasteels, 1961, 1965a). Since these reviews contain data on the early changes in fertilization without distinguishing between physiologically monospermic and physiologically polyspermic eggs and stress particularly the morphological differences and timing of cortical changes in different animals, it was impossible to generalize from the very diverse material presented. This fact and, in particular, the absence of distinct cortical changes in fertilized eggs of bivalve mollusks and the worm Chaetopterus led Pasteels to deny any biological importance to visible changes in the cortical layer of the fertilized egg.

The view of most embryologists that the bursting of cortical bodies is characteristic for fertilization and has general significance was interpreted by Pasteels as hasty and unverified conclusions from data on sea urchin eggs. According to Pasteels, the discharge of the cortical contents is an "epiphenomenon" of activation, i.e., an accompanying process which attracts attention but is in fact a negligible stage of the cortical reaction with importance only as mechanical protection. According to him, the block to polyspermy is based on a single mechanism operating at the molecular level, independent of visible cortical changes. This mechanism ensures monospermic fertilization in bivalve mollusks, whereas in other animals its action may be supplemented by the protective function of various formations such as the jelly coat and the hyaline layer in sea urchins, or the micropylar apparatus and cortical discharge in fishes.

Unlike Pasteels, I have found that the morphologically-expressed process of cortical secretion is the sole basis of the block to polyspermy in various animals, and not hypothetical transformations of the cortical layer at the molecular level. In my opinion, the importance of this process has been fully proven in animals (acipenserids, teleosts and sea urchins) in which the mechanism blocking polyspermy has been specially studied. It is also very likely that the block to polyspermy has a similar basis in many other animals whose cortical reactions resemble those of fishes and sea urchins, namely, certain polychaetes, starfishes, enteropneusts. Cyclostomi, urodele amphibians, mammals, etc. With respect to bivalve mollusks eggs, there are already grounds for believing that the differences between them and eggs of other animals having typical cortical reactions is not so very great (see p. 245).

To determine the prevalence of the mechanism blocking polyspermy based on cortical secretion, it is necessary to study the structure of the cortical layer and its changes during fertilization in various animal groups having physiological monospermy. With respect to many of these there are no data at all (e.g., sponges, cnidarians, some classes of flatworms and annelids, nemertines, bryozoans, etc.; see Table 31). Of special interest are those cases of monospermy in which the spermatozoon penetrates the oocyte even before completion of maturation, such as sponges Sycon and Grantia, turbellarians Mesostoma, Otomesostoma and Dinophilus, and the annelid Saccocirrus.

Variability of the mechanism blocking polyspermy

The differences in egg structure and conditions of gametic association imply that the mechanism blocking polyspermy has a given variability which is mainly connected with differences in the surface area in which spermatozoa can contact the ooplasm.

Teleost eggs have the greatest limitation of receptive surface; their eggs are enveloped by a membrane impermeable to sperm containing a single micropyle (whose end diameter is about equal to the width of the sperm head). The sperm-permeable surface is far greater in teleost eggs having several narrow micropylar canals. Next in the series, the sperm-permeable surface in eggs of Cyclostomi is limited to the base of the gelatinous protrusion at the animal pole. In urodele eggs this region is much wider (the entire animal hemisphere in frog eggs).

Apparently, the disruption of the usual relationships between gametic association and the cortical reaction observed in the polychaetes Sabellaria and Pomatoceros has the same significance. After spawning in water and having undergone the initial stage of the cortical reaction, eggs are permeable to sperm only at sections of the surface retaining of contact between the cytoplasmic filaments and the membrane.

In sea urchin eggs, there is merely a tendency toward limitation of the sperm-permeable surface, since some regions are more receptive than others.

Another quite important factor in preventing spermatozoa from penetrating is the discharge of substances from the egg, making spermatozoa infertile. In sea urchin eggs, this consists of egg water and of a solution of jelly coat substance identical to fertilizin. There are grounds for believing that these same factors operate in many other animals as well, including annelids, mollusks, starfishes, and chordate (Cyclostomi, fishes and urodele amphibians whose eggs discharge fertilizin (see Tyler, 1948).

The different variants in the mechanism blocking polyspermy apparently have equal biological value.

The simple and ideal mechanism blocking polyspermy in teleosts involving maximum limitation of the sperm-permeable surface, ensures monospermy even in optimal polyspermic conditions (high concentration of spermatozoa and poor physiological condition of the eggs). Such a limitation has a negative aspect, however, inasmuch as it can lead to a sharp drop in the fertilization percentage at low concentrations of spermatozoa (which can even occur in natural spawning).

The presence of several micropyles in acipenserids allows for polyspermic fertilization under conditions conducive to it, leading to the death of such eggs. However, this character has adaptive significance by increasing the probability of fertilization when eggs are laid in a rapid current. The mechanism blocking polyspermy in acipenserids must be sufficiently effective under natural spawning conditions; otherwise, the wide variation in numbers of micropylar canals would be limited by selection.

The sperm-permeability of the entire echinoderm egg surface is compensated by the protective function of the jelly coat and, perhaps, other factors reducing the probability of effective contact between several spermatozoa and the egg surface.

The failings of such a mechanism for blocking polyspermy appear only under abnormal conditions, such as a high concentration of spermatozoa, removal of the jelly coat or retardation of the cortical reaction. The percentage of polyspermy is negligible in eggs inseminated under optimal conditions.

6.2.5. Phylogenetical Reduction of the Polyspermy Blocking Mechanism in Response to Changes in Conditions of the Gametic Encounter

The experimental study of the mechanism blocking polyspermy has made it now possible to speculate on the phylogenetical changes in the type of fertilization.

As concluded above, monospermic fertilization is ensured by the cortical secretion with the participation of certain additional factors — limitation of the sperm-permeable egg surface, effect of substances produced by the egg. A harmonious combination of all elements of this mechanism is required to prevent polyspermy.

Such a combination, developed by natural selection, may be disrupted by a change in the conditions of gametic association. As a result, the egg is penetrated by a number of spermatozoa, deformities appear and the embryos die. Such disruption can be induced experimentally by increasing the concentration of spermatozoa above the adaptational limit for the given species, by removing some or all of the egg coverings, and by various chemical and physical agents changing the condition and sensitivity of the cortical layer.

Such changes were induced naturally in certain groups of higher animals (some arthropods, mollusks and chordates) when fertilization in water was replaced by the gametic association in the female genital tract. Such a change was usually associated with the emergence on dry land; however, even in the aquatic milieu it is biologically progressive. It increases the probability of fertilization, and in oviparous forms leads to wider possibilities for mutation and improvement of the tertiary egg membranes (which are impermeable to sperm over their entire surface); in addition this was an initial step toward interuterine development of the embryo.

With the transition to internal fertilization, a number of factors developed which disrupt the operation of the mechanism blocking polyspermy, as illustrated in the various cases below.

1. There was partial reduction of egg membranes, which previously hindered penetration of spermatozoa. The mechanical function of these membranes lost its initial significance with the development of strong tertiary membranes which reliably protect the fertilized egg from damage and desiccation. Rückert (1892b) indicated this factor as a possible cause of the appearance of physiological polyspermy in selachians.

2. Accumulation of large yolk stores and the corresponding increase in egg size was made possible by the protection against adverse external effects. These changes necessarily considerably prolonged the time required by the cortical reaction to spread over the egg surface. The significance of this time effect is confirmed by the close relation between egg size and type of fertilization, as already mentioned.

3. The high concentration of spermatozoa in the female genital tract exceeded their concentration in water.

4. Finally, gametic association involving not quite mature oocytes, whose cortical reaction is still incomplete, became possible.

These changes in egg structure and conditions of gametic association sharply reduced the efficiency of the mechanism blocking polyspermy; this was accompanied by a rise in the percentage of nonviable polyspermic embryos, which was detrimental to the species. Thus, the evolutionary transition to internal fertilization, involving disruption to the mechanism blocking polyspermy, led to adaptations which prevented development of the supernumerary spermatozoa at later developmental stages when the spermatozoa had already penetrated the ooplasm.

A new mechanism developed, under these conditions, on the basis of the plastic physiological properties of immature cytoplasm (see pp. 229–230).

Such phylogenetical changes in the type of fertilization can be traced in chordates. The eggs of primitive chordates — enteropneusts, Acrania, Cyclostomi — have cortical layers and, apparently, the single mechanism blocking polyspermy common to echinoderms and certain other invertebrates. This suggests that the original type of fertilization in chordates was physiological monospermy, especially since it is retained in higher groups of chordates (acipenserid and teleost fishes, urodele amphibians) with external fertilization. The transition to internal fertilization was consistently accompanied by loss of the mechanism preventing penetration of supernumerary spermatozoa and by the appearance of physiological polyspermy, as found in selachians and chimaeras, anuran amphibians, reptiles and birds. Mammals are a sole exception; despite the change in fertilization conditions they have retained the apparatus blocking polyspermy.

The following may be suggested for explaining this difference between mammals and other amniotes. Primitive reptiles retained the external fertilization inherent in their amphibious progenitors. The branch leading to extant reptiles later passed to a purely terrestrial existence and internal fertilization appeared in this group combined with improvement of the tertiary membranes and increased egg size. This led to a change from the original type of fertilization to physiological polyspermy. By contrast, the branch of primitive reptiles which evolved into mammals developed under semiaquatic life conditions (Olson, 1965), so that fertilization remained external in this group. The transition to internal fertilization in the mammalian progenitors, combined with progressive adaptations ensuring the nutrition of the embryo by the maternal organism. Accordingly, the egg size did not increase and the oocyte membrane was not reduced in this group, making retention of the original type of fertilization (physiological monospermy) possible.

SUPPLEMENT

PRACTICAL APPLICATION OF THE DATA ON FEATURES OF GAMETES AND PATTERNS OF FERTILIZATION

In the breeding of acipenserids, salmonids, pikes, Rutilus frisii kutum and certain other fishes, all steps in the reproduction process, beginning with mature sexual products and insemination, are in the hands of the fish breeder. Elaboration of biotechnics for obtaining and storing sexual products and rational artificial insemination must be based on a knowledge of the properties of the eggs and spermatozoa of these fishes, the conditions in which the fertility of eggs and spermatozoa is preserved longest, as well as of fertilization patterns. Information on these questions has been presented in the respective sections of the book. Now, a brief summary of what is significant to fish breeding will be given, together with some additional data.

Preservation of Eggs

Best results are obtained by inseminating eggs immediately after they are obtained. If this is impossible and unfertilized eggs must be stored for a certain period, they should be kept in their cavity fluid since the fertility of all eggs studied was retained much longer in cavity fluid than in water or other media (Table 16, Figures 19 and 20).

Eggs from an anaestisized female remain fertile for a certain time, but this period is considerably shorter than that in stored, stripped eggs (see, for example, Stroganov, 1936a; Blaxter, 1955).

Obtaining and Storing Sperm

The sperm of different males of a single species, whether acipenserid or teleost, can vary greatly in its properties.

1. Similar volumes of sperm contain various numbers of spermatozoa. The concentration of spermatozoa in ejaculates varies by 2–3 times, sometimes by several dozen times. For example, the number of Acipenser güldenstädti colchicus spermatozoa per cc of ejaculate varies from 0.14 to 7.55 $\times 10^9$ (Table 21).

2. The percent of spermatozoa becoming motile when the ejaculate is diluted with water varies considerably; in some males all or most of the spermatozoa become motile, in others a small part of the spermatozoa are activated and sometimes all remain immotile (see p. 139).

3. The time in which spermatozoa retain fertility when kept in water differs greatly from that when stored as "dry" sperm (see pp. 139–141, 144).

All these variations are not only expressed among different males of the same species, but also in the same male whose sperm is sampled twice.

Because of the variable sperm quality, a mixture of sperm from several males, preferably 3–4, should be used.

If there is a shortage of males and, as often happens, one is restricted to the sperm of a single specimen, its quality must be verified microscopically before insemination. It is necessary that a sufficiently high percentage of spermatozoa acquire motility when water is added, since only such spermatozoa can fertilize eggs.

Spermatozoa remain fertile longest in seminal fluid where they remain immotile. To preserve their fertility for a long time, the sperm should not only be kept inactive, but should be protected by the seminal fluid (like eggs in cavity fluid). Any medium stimulating movement causes loss of fertility within a relatively short time, most rapidly in freshwater. When milt is stripped, care should be taken that no water enters the sperm and it then should be stored as "dry" sperm. The ideal temperature range for storing "dry" sperm is between 0° and 4°C (Table 26).

Storing of a spawn mixture from several males leads to a more rapid decline in its fertility than when the sperm of each male is stored separately.

Preservation of entire gonads, removed from the male, can give good results. Thus, spermatozoa in testes of Clupea harengus harengus (Blaxter, 1955) kept at 4°C were still highly fertile after 2 days, and even after 8 days were capable of fertilizing about 12% of the eggs. When kept at a somewhat higher temperature (7°C), fertility declined in the first few days and was completely lost on the 7th day. If the gonads were left in the body of dead mature fishes, 50% of eggs were fertilized after 7 hours and only about 3% after 27 hours.

In experiments on Alosa volgensis (Stroganov, 1936a), spermatozoa from a dead male kept at 26°C died within the first hour, whereas collected sperm kept at the same temperature retained motility for up to 30 hours.

There are no comparative data on retention of fertility in collected sperm and spermatozoa in dissected testes.

Method of Insemination

Different methods of artificial insemination — wet, semidry, and dry — have long been used in breeding of various fishes. The choice of insemination method is often made without taking into account the specific properties of the gametes of the given species, but depends on the personal experience of the fish breeder or the tradition in the given fish breeding organization.

In his handbook on fish breeding in natural and artificial water bodies A. N. Eleonskii (1936, p. 56) writes as follows: "It must be noted that the question of application of one method or another to this or that kind of fish has still not been clarified owing to lack of scientific observations; it can only be guessed that eggs of different species require different methods of artificial insemination considering their different biological properties."

Teleosts. At first, artificial insemination of eggs of salmonids and certain other teleosts was carried out using the wet method of insemination which most nearly reproduces conditions of the gametic encounter in nature. Eggs of a spawning female were collected in a vessel containing water, while milt was collected in another vessel containing water; the sperm solution was then added to the eggs (Grimm, 1931; for an account of the development of artificial insemination methods see Skatkina, 1962). This method of insemination gave inconsistent and often quite unsatisfactory results, since the spermatozoa often became infertile before the sperm solution could be added to the eggs, and by this time there was also often a decline in the eggs' fertility.

The special characteristic of gametes of salmonids and certain other teleosts, namely, the very short period in which they retain fertility in water, was first taken into account by the distinguished Russian fish breeder Vladimir Pavlovich Vrasskii. This fact served as a basis for a new insemination method, called at the time the "dry fertilization method", but named later the semidry method (Rul'e et al., 1857; Vrasskii, 1859). In this process, eggs and sperm were collected separately in dry vessels; the sperm was diluted with water only immediately before being added to the eggs. In the case of salmonids this method invariably gave a high fertilization percentage. Later it was modified as follows: running sperm was poured directly onto the dry eggs, then mixed and after that a little water added (Grimm, 1931).

This modification of Vrasskii's method, named the dry or Russian method of insemination, has spread throughout the world and universally superseded the wet method for breeding of salmonids and other teleosts (Guénaux, 1923; Davis, 1956; Shelbourne, 1964).

At present, however, the dry method of insemination is not regarded as optimal for all teleost species. A knowledge of the properties of the sexual products of various fishes makes it possible to determine where it can be applied.

Use of the dry method is justified for species in which the spermatozoa are fully activated in the cavity fluid, such as various species of trout, salmon, Coregonus muksun, grayling and silver salmon. Gametic association in these cases occurs in a medium ensuring long retention of spermatozoan motility and fertility even before water is added.

The considerable advantage of the dry method of insemination over other methods for this group of fishes was graphically demonstrated by our experiment on lake trout (see Figure 67; Ginzburg, unpublished). The dry method gave a high percentage of fertilized eggs in all sperm dilutions, whereas the semidry method gave a high percentage only with concentrated solutions of spermatozoa (dilutions of 1:100 and 1:9); the wet method (insemination of eggs after sperm was kept in water for 30 seconds) always resulted in fertilization of only a small percentage of eggs.

When inseminating eggs of certain species of this group, for example, rainbow trout (Medem et al., 1949) and grayling (Roth et al., 1950), it is necessary to take care that water does not enter the vessel with the eggs, since spermatozoa of these fishes immediately agglutinate in aqueous solutions of cavity fluid, causing a sharp drop in the fertilization percentage (agglutination does not occur in undiluted cavity fluid).

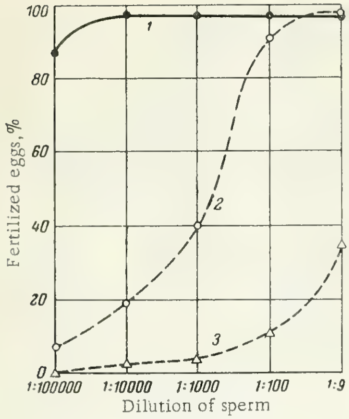

FIGURE 67. Insemination of lake trout eggs by various methods: dry (1), semidry (2) and wet with a single washing of cavity fluid in 30 seconds (3).

Temperature 7.8°C, concentration of spermatozoa in ejaculate 1.19 × 10^{10} per cc.

For teleosts whose spermatozoa are rather inactive in cavity fluid, such as whitefish and pike, the dry method of insemination must be somewhat modified. Maximum motility and retention of fertility of spermatozoa are attained in a mixture of cavity fluid with water. It is best to add an equal volume of water to the gametes soon after collecting in cavity fluid and to mix thoroughly (Medem et al., 1949; Roth et al., 1950). Such a modification of the dry method is applicable only to fishes, such as whitefish and pike, in which dilution of cavity fluid with water does not cause agglutination of the spermatozoa.

Finally, spermatozoa of a number of teleosts (Alosa volgensis, loach, Diptychus, Schizothorax, Rutilus frisii kutum, Vimba vimba) are not activated at all in cavity fluid. The dry method of insemination may be less suitable for these fishes than the semidry and wet methods; for insemination it is only necessary that the gametes should be kept in water for a minimal time before mixing.

For insemination of Alosa volgensis eggs, both the dry and wet methods apparently give equally good results, i.e., 90–100% fertilization (Stroganov, 1938b). In Vimba vimba, as in Alosa, spermatozoa are not activated in cavity fluid and lose their fertilizing capacity in water very rapidly (within 15–30 seconds), making a modified wet method of insemination undoubtedly more suitable. Eggs and sperm are collected separately in dry vessels, simultaneously poured into water and mixed (Smirnova and Kuz'mina, 1966). Two experiments of this insemination method gave somewhat better results than the semidry method (fertilization rate was 4–5% higher) and considerably better results than the dry method (the difference in fertilization rate was 18–21%).

Acipenserids. Until recently, all three methods of artificial insemination were used in sturgeon breeding.

The semidry method was introduced in sturgeon breeding at the end of the 19th century by N. A. Borodin, who performed his first artificial insemination experiments on eggs in the Ural River. A state conference on fish breeding in 1915 (1916) recommended this method for inseminating sturgeon eggs. However, the semidry method was subsequently not widely used. In the 1950s it was successfully employed together with the dry method by P. A. Samsonenko at the Rogozhkinskii sturgeon breeding station. A similar method was suggested by I. A. Sadov for inseminating eggs in a trough-type apparatus (Sadov, 1953, 1957).

TABLE 37. Comparison of various methods of artificial insemination of sturgeon eggs

Species	Experiment no.	Temperature, °C	Quantity of sperm per kg eggs in cc	Dilution of sperm	% fertilization		
					dry method	semidry method	wet method
Huso huso	1	12.9	20	1:200	41.9	93.2	81.9
Acipenser güldenstädti	2	11.4	8	1:500	19.7	93.5	90.7
	3	20.8	20	1:200	39.1	90.1	88.5
	4	19.2	20	1:200	65.2	93.4	92.1
	5	19.6	20	1:200	6.6	65.8	59.6
	6	18.1	40	1:100	88.4	87.9	75.0
	7	14.5	40	1:100	82.7	92.7	89.5
	8	16.8	40	1:100	94.8	94.0	76.6
	9	13.1	80	1:50	78.3	95.1	91.6
Acipenser stellatus	10	13.6	4	1:1000	80.8	90.4	52.0
	11	17.0	8	1:500	56.6	57.3	24.8
	12	19.1	20	1:200	93.2	97.1	69.2
	13	16.6	40	1:100	74.7	94.8	85.8

In the 1960s, A. P. Derzhavin employed a somewhat modified wet method for inseminating sturgeon eggs; before insemination the eggs were thoroughly washed in water to remove the viscous cavity fluid in which they were immersed; then sperm was added to the water. This method later gave satisfactory results in the Kura and Volga rivers (Maslov, 1919). It was employed at the Kurinskii sturgeon breeding station until recently (Guseinov, 1959).

The dry method of insemination has been used for many years at most sturgeon breeding stations and hatcheries. This method was recommended in 1952 in an instruction manual issued by the Main Administration for Fish Breeding and Conservation (Vernidub, 1952).

A comparison of the efficiency of the three insemination methods, carried out under commercial conditions at the Rogozhkinskii sturgeon hatchery (Ginzburg, 1963d; Ginzburg et al., 1963), showed them to be far from equal.

From results of the experiments given in Table 37 it can be seen that the semidry method of insemination invariably gave higher fertilization percentages than the other methods used.

The dry method produced a sharp deterioration in insemination results in Huso huso and Acipenser güldenstädti eggs when smaller amounts of sperm were used (experiment nos. 1–5 in which 8 and 20 cc were used per kg eggs); 28–74% less eggs were fertilized than with the semidry method. It should be remembered that such amounts cannot be considered insufficient. The manual cited above (Vernidub, 1952) recommends adding "not less than 4–5 cc sperm per kg eggs." When very large amounts of sperm were used (40–80 cc per kg eggs), the variation was smaller: (10–17%; experiment nos. 7 and 9) or virtually nil (experiment nos. 6 and 8).

The wet method gave better results than the dry method, but somewhat poorer results than the semidry method.

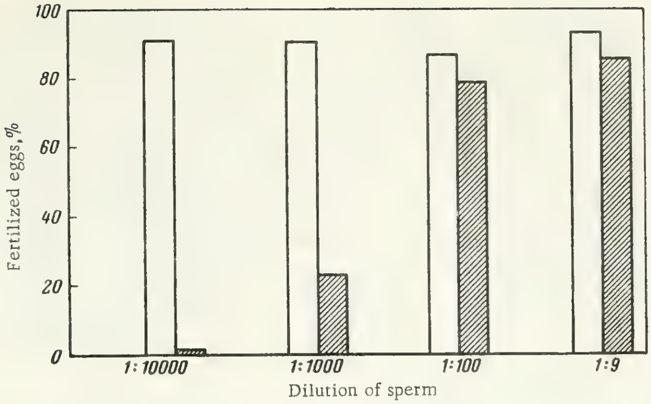

FIGURE 68. Fertilization percentage of sturgeon eggs inseminated with sperm diluted with water (white columns) or with sperm mixed with 10 cc cavity fluid before dilution with water (hatched columns). Temperature 16.0°C.

The difference in Acipenser stellatus eggs between dry and semidry methods was less than with Huso huso and Acipenser güldenstädti. On the other hand, the wet method caused a sharp drop in fertilization percentage not only compared with the semidry method but usually also the dry method. A study of egg samples indicates that this decline in fertility is due to activation of unfertilized A. stellatus eggs, as well as direct damage when eggs are washed free of cavity fluid.

The above comparison indicates that the best and most uniform results for sturgeon eggs are obtained using the semidry method, which is far more effective than the dry method for Huso huso and Acipenser güldenstädti and more effective than the wet method for A. stellatus eggs. The differences are smaller in comparison with the wet method for Huso huso and A. güldenstädti or the dry method for A. stellatus, but here too the semidry method proved best.

The dry method of insemination is less suitable for sturgeons not only because spermatozoa are not activated in cavity fluid, but this fluid hinders activation in water as evident from the following experiment. Small batches of A. güldenstädti and A. stellatus eggs were inseminated with equal amounts of sperm; in the first series the sperm was diluted with water, while in the second it was mixed first with 5 or 10 cc cavity fluid and then water was added. It was found (Ginzburg et al., 1963, unpublished data; see Figure 68) that the fertilization percentage was regularly lower in the second series. The difference was particularly great with small amounts of sperm.

The adverse effect of cavity fluid on eggs inseminated by the dry method appears strongly in Huso huso and Acipenser güldenstädti whose eggs often have a large amount of viscous cavity fluid; it appears least in A. stellatus eggs in which the cavity fluid is usually less copious and viscous.

The wet method may be used in sturgeon breeding because the eggs retain fertility relatively long in water (see Figure 20). However, the semidry method consistently gives better results. There are two reasons for this: first, when the wet method is used, some eggs may be activated before insemination (as a result of mechanical effect of washing in cavity fluid and directly by the water) and become infertile; secondly, the activated eggs present in a certain proportion in each batch (Detlaf and Ginzburg, 1951, 1954) become sticky in the water and can form clumps. None of this can significantly affect the results of insemination in egg batches with a weak tendency toward activation. This is usual with Huso huso and Acipenser güldenstädti, although the fertilization percentage is reduced in individual cases. In experiments of A. stellatus, such a decline is generally observed and clumps are readily formed, rendering the eggs unsuitable for breeding purposes.

When applied to sturgeon eggs the wet method can eliminate the adverse effect of cavity fluid on the spermatozoa. The semidry method has the same advantage, while lacking the defects of the wet method. With the semidry method, eggs are immediately fertilized on entering the water, therefore, (1) there is no additional activation of eggs by water and (2) eggs activated in the female body cavity become sticky at the same time as fertilized eggs, but do not stick together with them and form no clumps.

Thus the superiority of the semidry method, indicated by a comparison of the various insemination methods, is quite consistent and depends on the biological features of the eggs and sperm of the acipenserids.

Sperm Concentration for Artificial Insemination of Fish Eggs

The calculation of norms for the amount of sperm required to achieve maximal fertilization of acipenserid and teleost eggs must consider features of the mechanism blocking polyspermy in these groups.

While the mechanisms blocking polyspermy in acipenserids and teleosts are very similar, important differences also exist between them.

The definite limitation of the sperm-permeable surface (a single micropyle in the dense egg membranes) in teleost indicates that the mechanism blocking polyspermy is reliable no matter what concentration of spermatozoa surround the egg. To determine the amount of sperm required to fertilize all the eggs, it is sufficient to establish the minimal spermatozoan concentration giving this result. Excessive sperm at insemination cannot have adverse effects.

This does not apply to acipenserids in which the sperm-permeable egg surface is less clearly limited (there are numerous micropylar canals in the egg membranes), so that eggs inseminated with highly concentrated sperm are often penetrated by several spermatozoa simultaneously. Polyspermy causes serious disruption of development and early death of the embryos (pp. 187–188, 199). Experimental insemination of eggs with sperm of various concentrations showed that the frequency of polyspermy may already be very considerable (up to 17%), when sperm is diluted with water at a ratio of 1:50,

TABLE 38. Insemination of sturgeon eggs by the semidry method using various dilutions of sperm

Species	Experiment no.	Dilution of sperm						Dilution of sperm					
		1:1000	1:500	1:200	1:100	1:50	1:20	1:1000	1:500	1:200	1:100	1:50	1:20
		% fertilization						% polyspermic eggs					
Huso huso	1	45.6	44.2	—	68.5	77.8	—	0.7	0.4	—	0.4	2.8	—
Acipenser güldenstädti	2	—	94.2	93.8	—	94.8	—	—	2.2	1.4	—	7.1	—
	3	95.1	93.9	—	96.1	—	91.8	2.6	2.5	—	7.3	—	20.1
	4	82.7	82.4	90.0	86.0	—	87.7	0.8	0.2	1.7	2.5	—	5.4
	5	73.7	84.1	98.0	97.7	—	96.0	1.0	1.0	6.9	6.0	—	20.7
	6	98.4	98.3	99.4	98.8	—	98.9	4.6	3.5	5.0	7.7	—	16.2
	7	96.0	94.5	94.4	—	96.9	96.8	3.6	4.6	9.1	—	16.9	19.0
Acipenser stellatus	8	—	59.7	64.0	62.5	64.9	—	—	3.2	5.7	7.8	16.1	—
	9	41.8	82.1	—	78.5	—	80.2	0.2	2.3	—	5.4	—	14.0
	10	91.2	93.1	87.7	—	85.3	—	1.7	2.4	5.4	—	10.9	—
	11	96.7	97.6	96.7	—	96.8	—	0.7	2.3	2.2	—	6.6	—

and that it reaches 20% in half of the experiments at a dilution of 1:20 (Ginzburg et al., 1963).

These results indicate that the optimal sperm dilution for inseminating sturgeon eggs is 1:200; this ratio ensures a high total fertilization percentage, whereas the frequency of polyspermy remains relatively low.

Insemination norms and procedure have been worked out on the basis of the data concerning the efficiency of the various methods of inseminating sturgeon eggs and the significance of spermatozoan concentration for insemination results. An account of these is given in a handbook on instructions for artificial insemination of sturgeon eggs (Ginzburg, 1963d); work at sturgeon hatcheries is now carried out according to these instructions.

PLATES

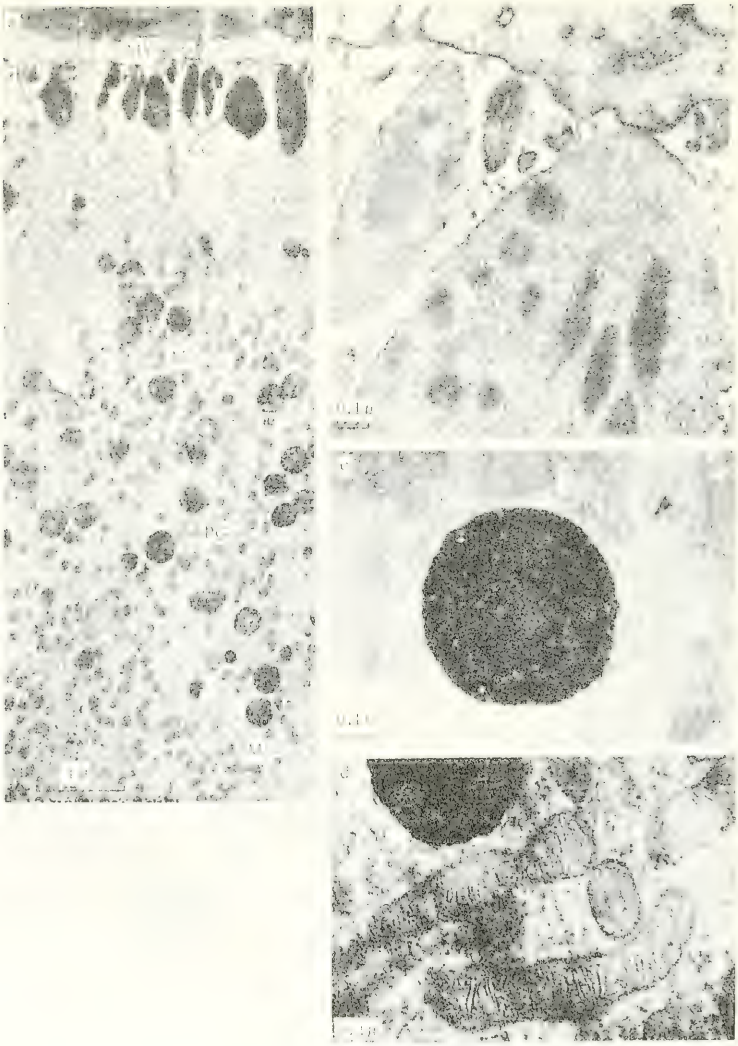

PLATE I. Surface layer of mature unfertilized sturgeon egg in the area of the animal pole (electron micrograph; Palade fixation, embedding in methacrylate, contrasting by uranyl acetate and lead citrate according to Reynolds):

a, c, d — Acipenser güldenstädti; b — A. stellatus. a — General view; b — cortical granules; c — pigment granule; d — mitochondria. CG — cortical granules; M — mitochondria; MV — microvilli; PG — pigment granules.

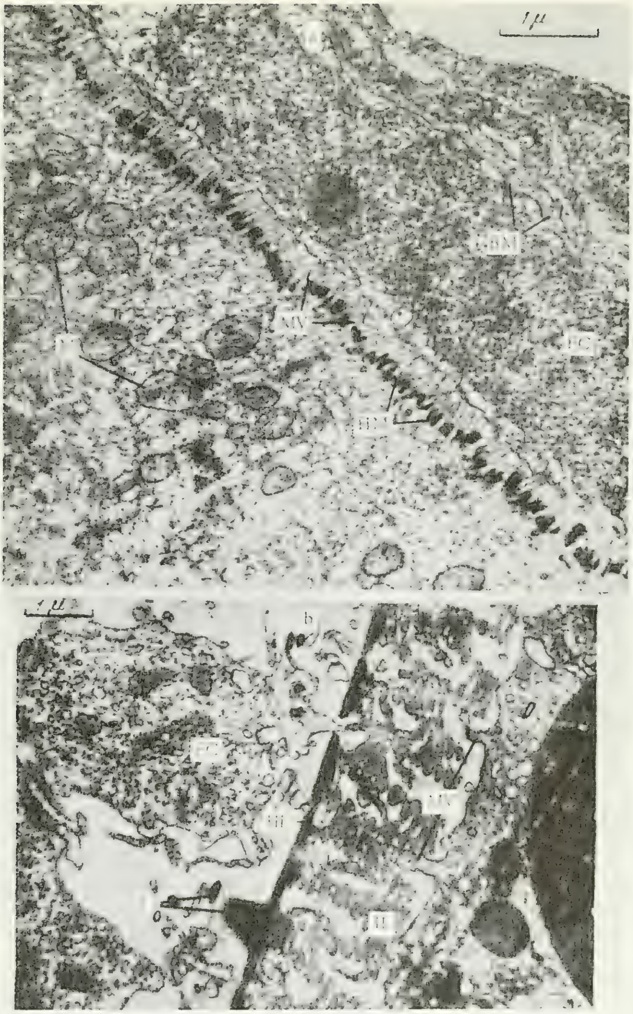

PLATE II. Egg membrane formation in Cynolebias belotti. Radial sections of oocyte margin and follicular epithelium:

a — previtellogenesis stage; b — stage of advanced vitellogenesis (after Müller and Sterba, 1963). BM — basal membrane; E — excrescence of homogeneous layer; IL — inner layer of zona radiata; HM — homogeneous material; HL — homogeneous layer of zona radiata; M — mitochondria; MV — microvilli; O — ooplasm; FC — follicular cell.

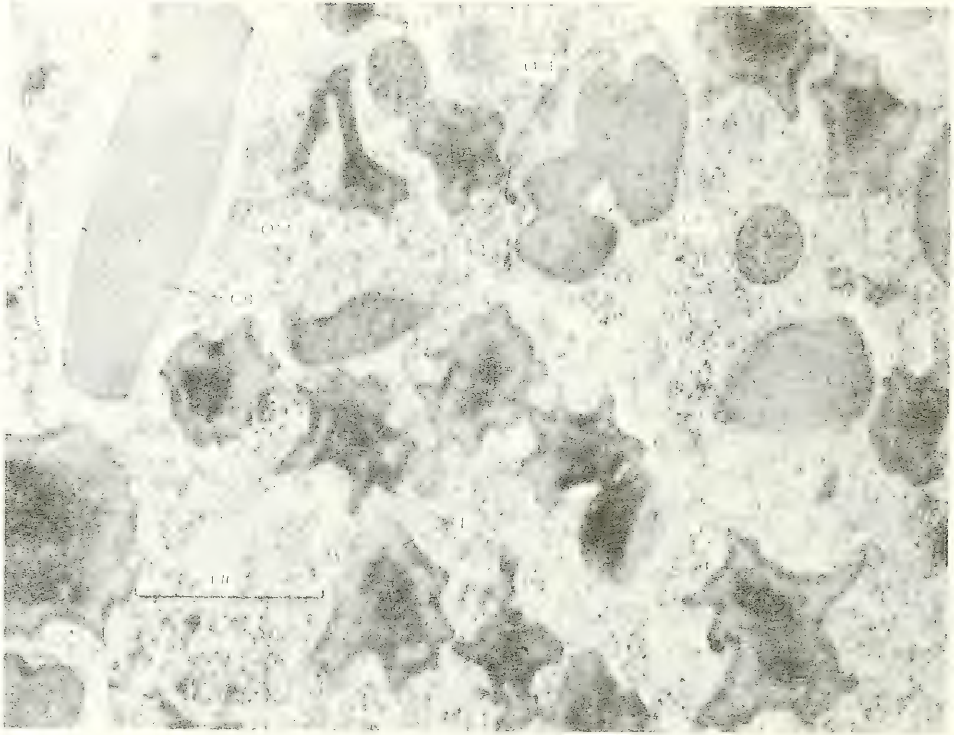

PLATE III. Yolk inclusions in animal pole of Acipenser stellatus egg. (Electron micrograph; treatment as described in the legend of Plate I):

L — lipid inclusions; M — mitochondria; OM — outer membrane of yolk granule; CB — central body of yolk granule.

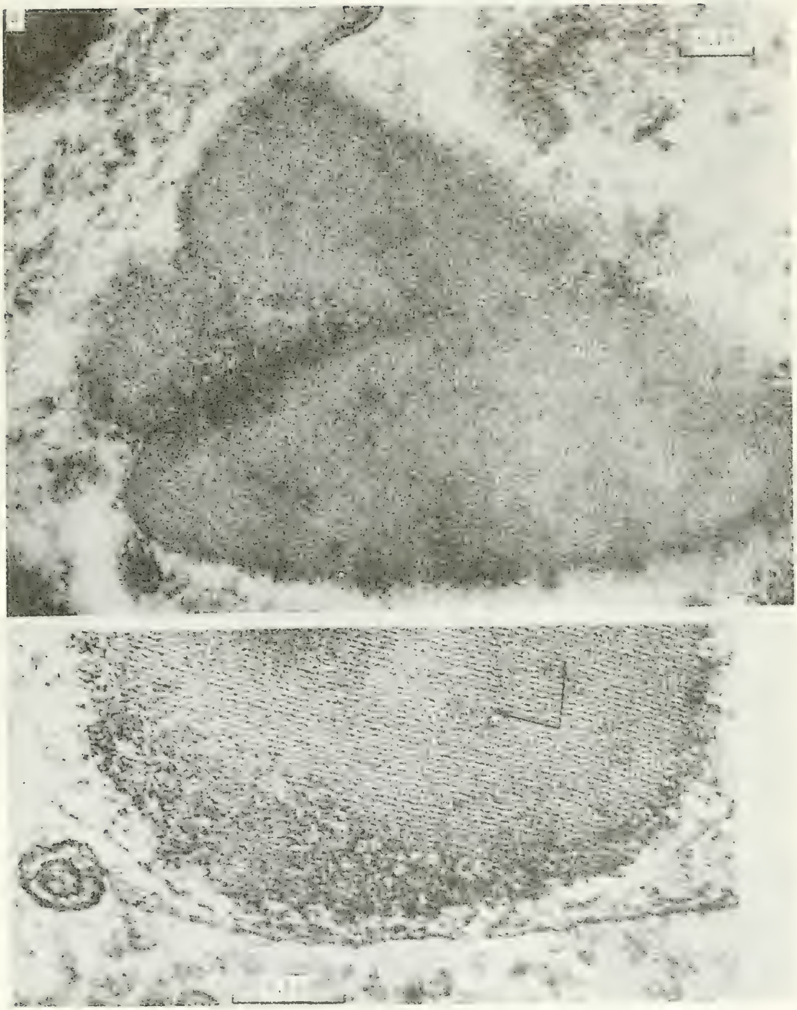

PLATE IV. Structure of yolk granules of Acipenser stellatus (electron micrograph treatment as described in legend to Plate I). The crystalline structure of the central body of the yolk granule can be discerned.

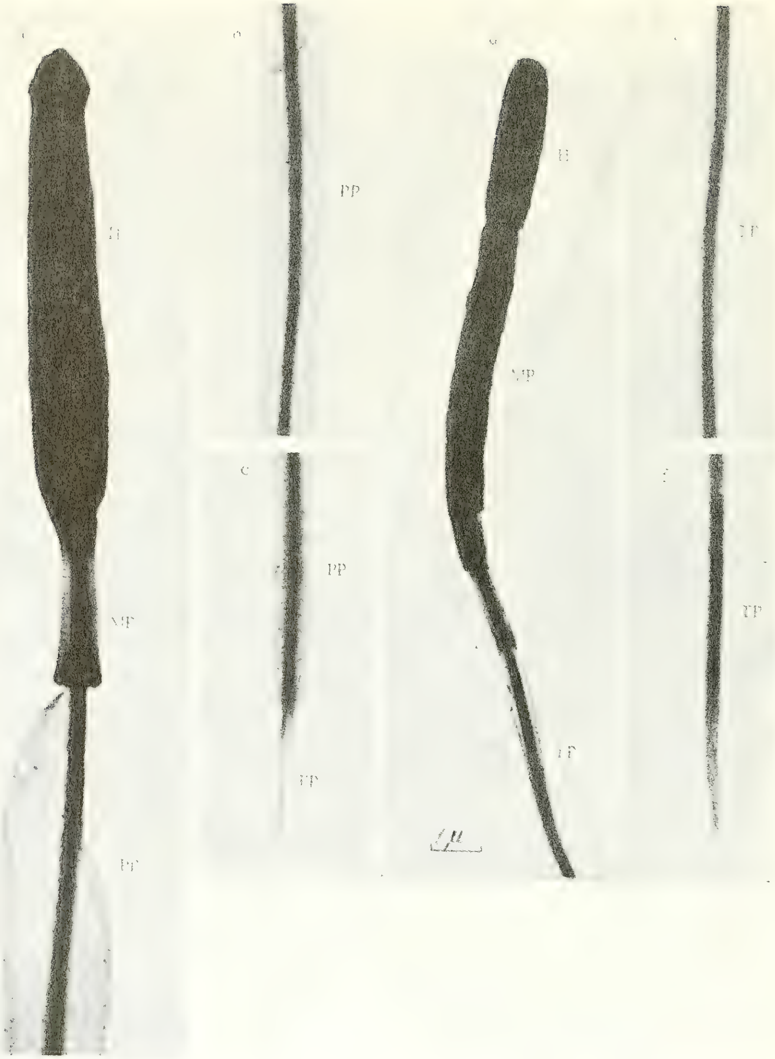

PLATE V. Spermatozoa of Acipenser güldenstädti colchicus (a–c) and swordtail (d–f) (electron micrographs; Palade fixation):

H – head; PP – principal tail piece; EP – end tail piece; MP – middle piece; TP – tail piece.

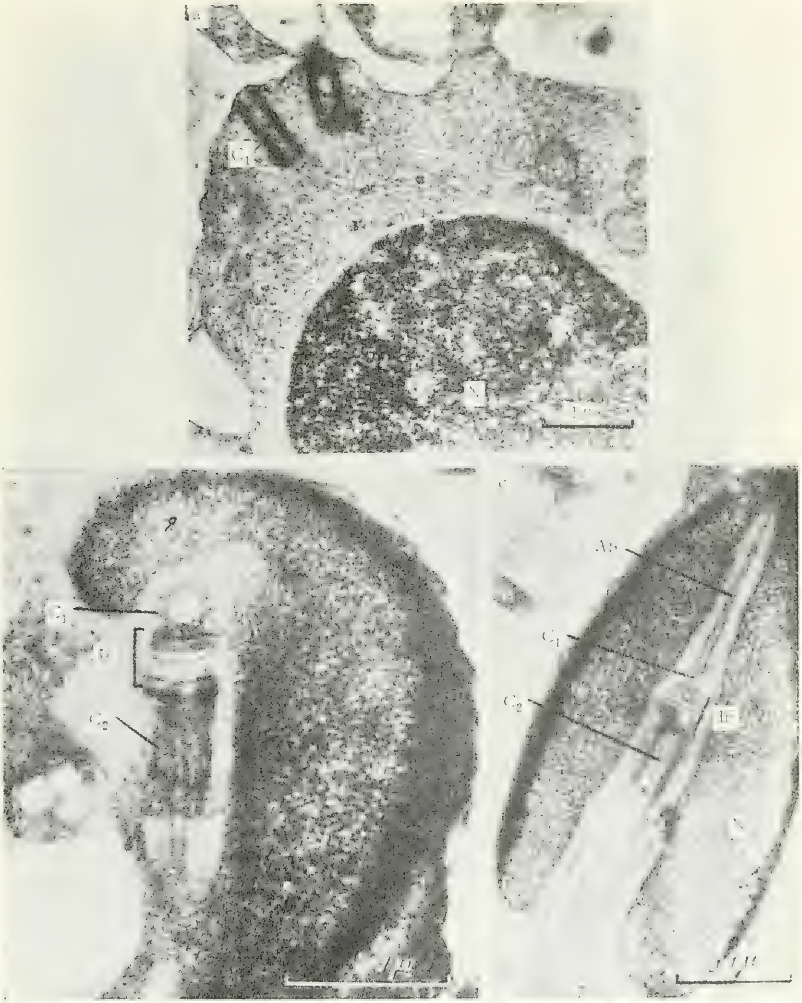

PLATE VI. Formation of middle piece during spermatogenesis in Poecilia reticulata:

a — young spermatid with two centrioles near cell membrane; b — spermatid with centriole complex in nuclear depression; c — late spermatid with completed axial body (after Mattei and Boisson, 1966). IF — intercentriolar formation; AB — axial body; C — centriole of young spermatid; C_1 — proximal centriole; C_2 distal centriole; N — nucleus.

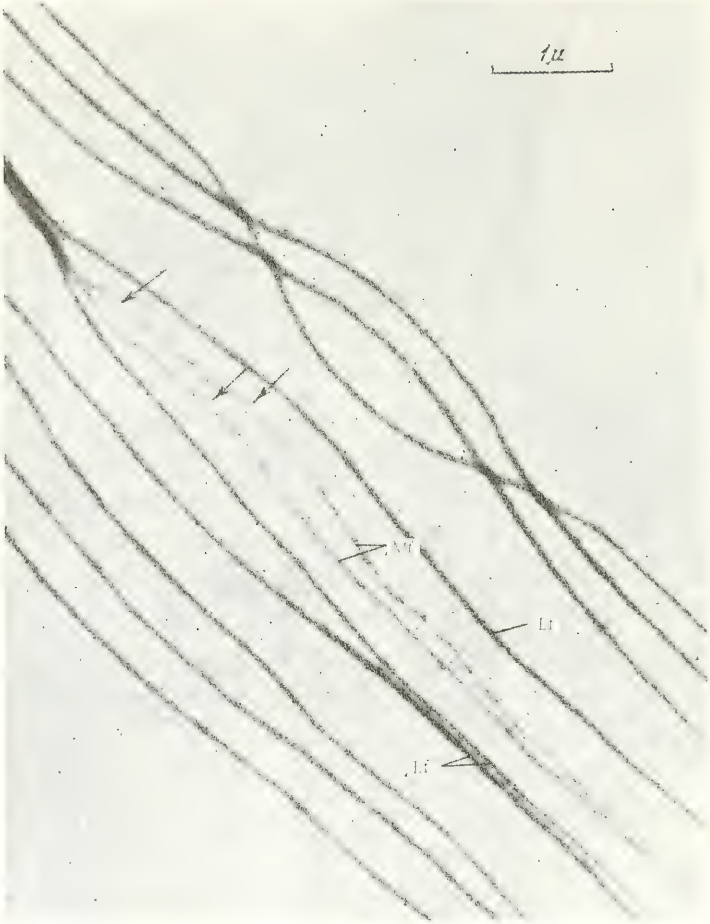

PLATE VII. Flagellal fibrils of Misgurnus fossilis spermatozoon after maceration in distilled water (electron micrograph):

Lf — peripheral L-fibrils; Mf — central M-fibrils. Arrows indicate points of breakup of M-fibrils.

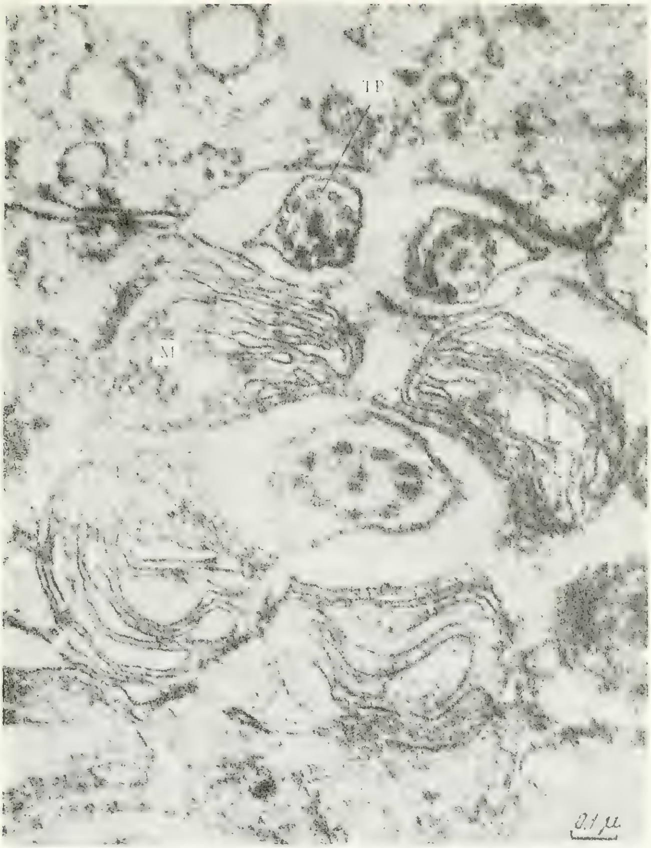

PLATE VIII. Middle and tail pieces of swordtail spermatozoon (electron micrograph of testical section):

M — mitochondrial body of middle piece; TP — tail piece.

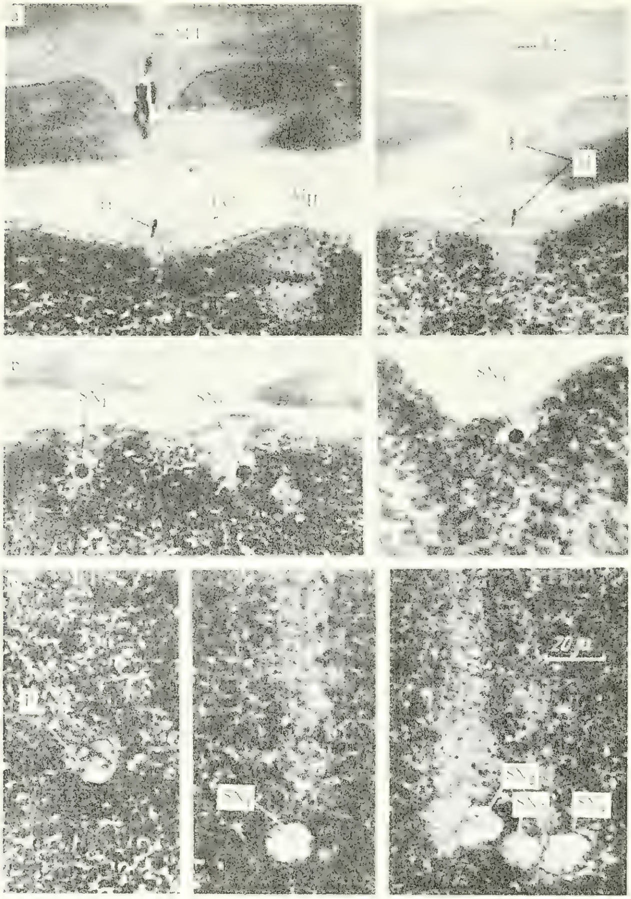

PLATE IX. Multiple penetration of spermatozoa to *Acipenser güldenstädti*:

a — 5 min after insemination, one spermatozoon has penetrated the egg near the maturation spindle (a second was a short distance away, in another section); b — 15 min after insemination, 3 spherical compact male pronuclei are seen in the egg; c — 60 min after insemination, female pronucleus and 4 vesicular male pronuclei deep in the cytoplasm (after Ginzburg, 1957a). FC — fertilization cone; H — sperm head; M_{II} metaphase of second maturation division; MT — micropylar tubule; P — female pronucleus; SN_1—SN_4 — male pronuclei.

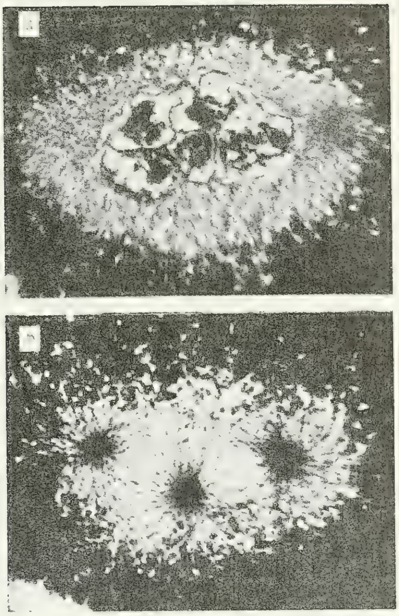

PLATE X. Fusion of several male pronuclei with female pronucleus in polyspermic egg of Acipenser güldenstädti:

a — group of fusing pronuclei; b — seminal asters surrounding the group of pronuclei, in adjacent microscopic section.

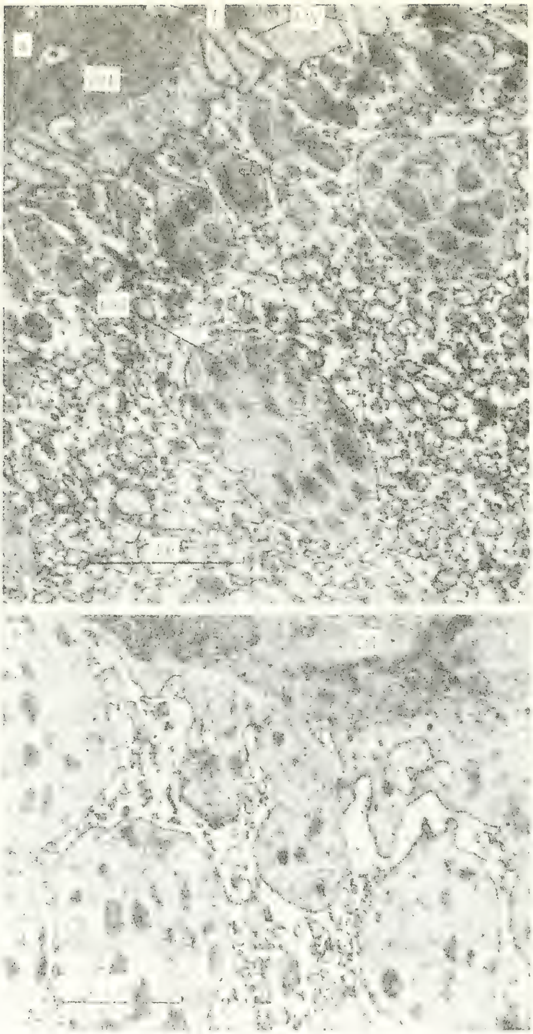

PLATE XI. Cortical reaction in Acipenser stellatus egg (17.7°C) (electron micrograph). Cortical layer of egg before fertilization (a), 5 (b), 30 (c) and 120 (d) sec after insemination (treatment according to legend of Plate I).

T — tubule zona radiata; CG — cortical granules; MV — microvilli; ZrI — inner layer of zona radiata.

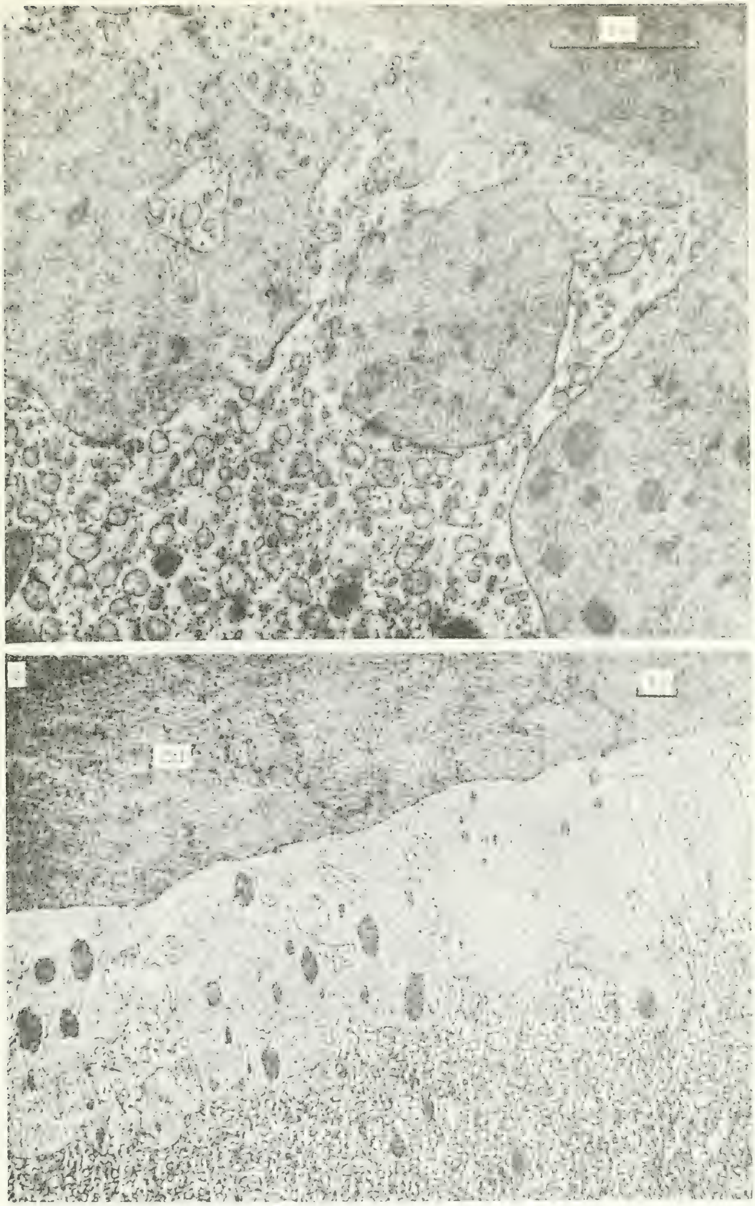

PLATE XI (Continued)

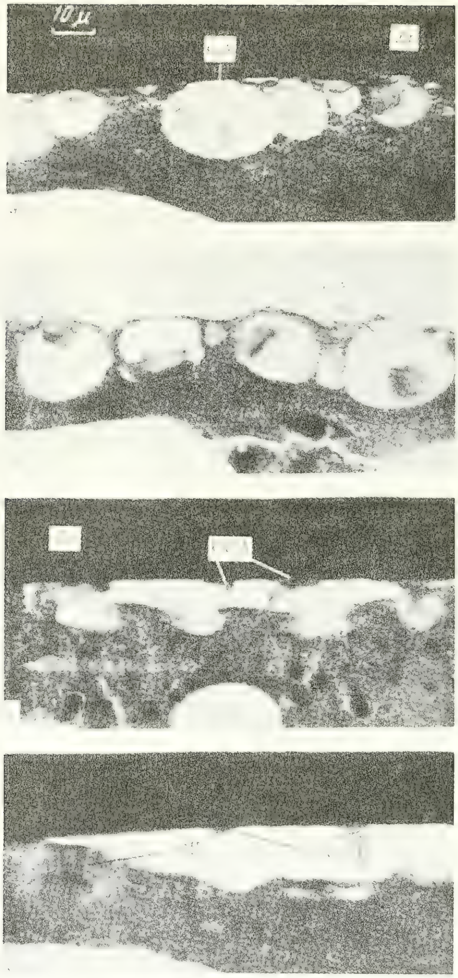

PLATE XII. Cortical reaction near micropyle of lake trout eggs activated by water:

Mature egg, before activation (a), 60 (b), 80 (c), and 120 (d) sec after activation at 40°C (after Ginzburg, 1960b). CA — cortical alveoli; M II — metaphase of second maturation division; CCA — contents of cortical alveoli; Zr — zona radiata.

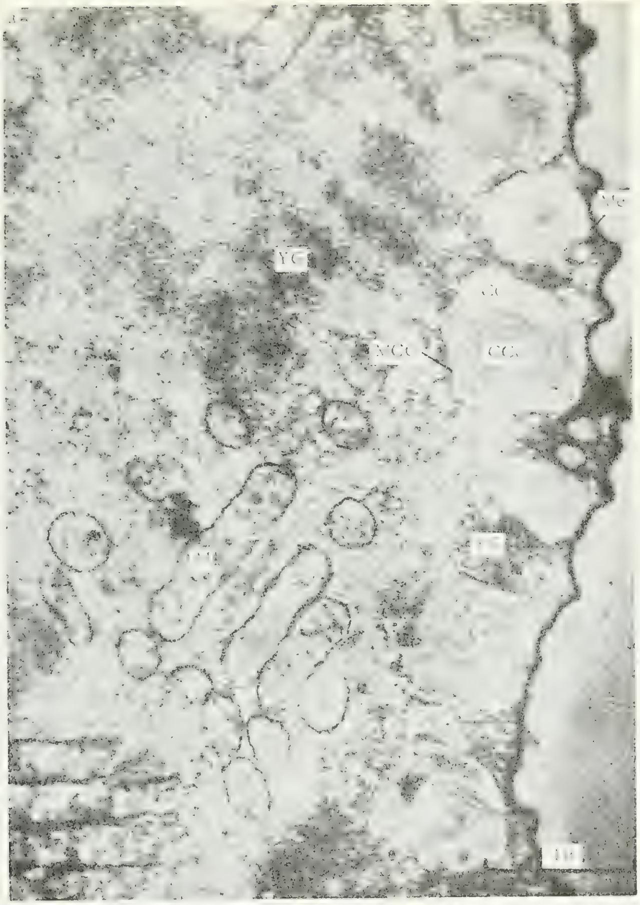

PLATE XIII. Discharge of cortical granule contents in egg of Psammechinus miliaris (electron micrograph):

a — surface of unfertilized egg; b — beginning of cortical granule discharge (lower part of granule has not yet burst and in places marked with arrows the egg membrane has split into the vitelline membrane and plasmalemma); c — vitelline membrane fully separated, cortical granule contents visible on its inner surface (after Wolpert and Mercer, 1961). YG — yolk granule; VM — vitelline membrane; CG — cortical granule; M — mitochondria; Me — surface membrane of unfertilized egg; MCG — membrane of cortical granule; FM — fertilization membrane; P — plasmalemma; CCG — contents of cortical granules.

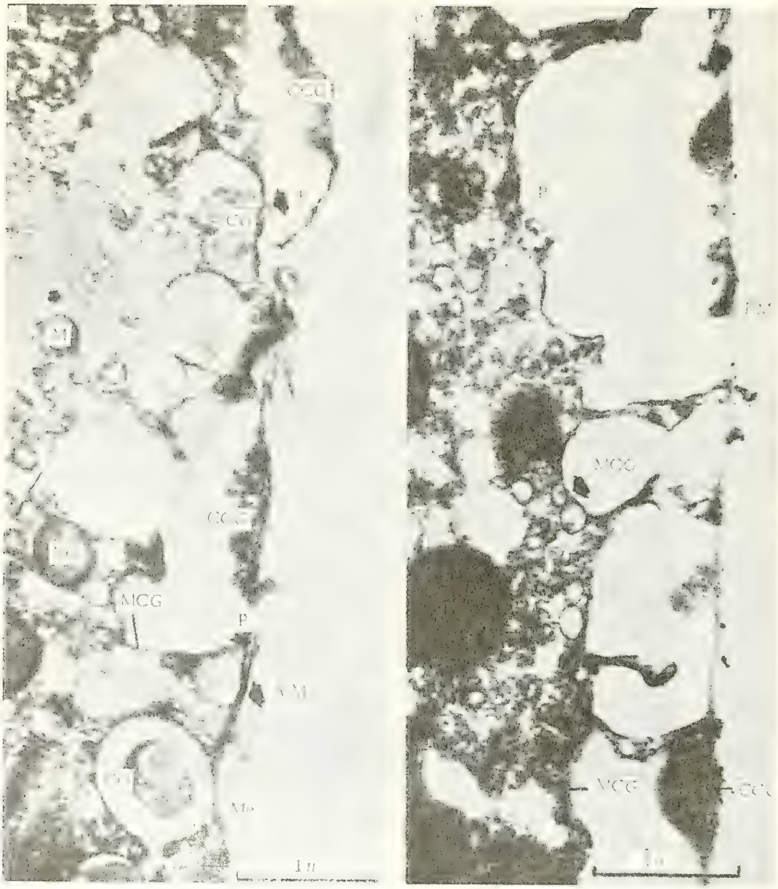

PLATE XIII (Continued)

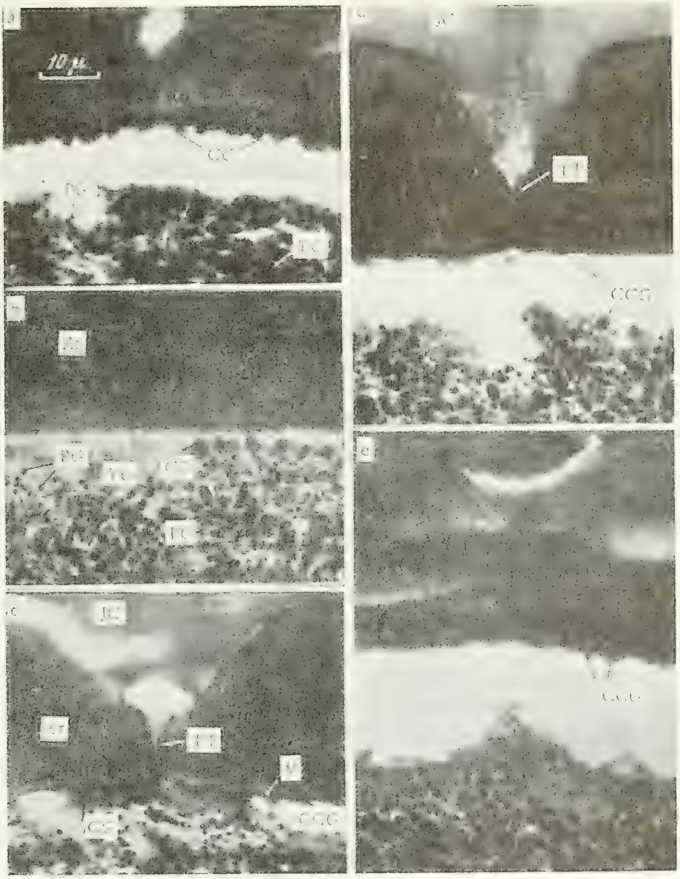

PLATE XIV. Cortical changes in Acipenser güldenstädti eggs in micropylar region at different times after their activation by pricking with a needle (13.8°C):

Five sec (a) after pricking near animal pole; 2 min (b), 3 min (c, d), and 4 (e) min after pricking in vegetal pole. Fixation in Sanfelice fluid, azocarmine staining according to Heidenhain (after Ginzburg, 1961a). V — vacuoles at point of discharge of cortical granule contents; YG — yolk granules; CG — cortical granules; TT — terminal tubule of micropyle; PG — pigment granules; CCG — contents of cortical granules; JC — jelly coat; Zr — zona radiata.

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EXPLANATORY LIST OF ABBREVIATED NAMES OF USSR INSTITUTIONS APPEARING IN THE BIBLIOGRAPHY

Abbreviation	Full name (transliterated)	Translation
LGU	Leningradskii Gosudarstvennyi Universitet	Leningrad State University
MOIP	Moskovskoe Obshchestvo Ispytatelei Prirody	Moscow Society of Naturalists
SNKh	Sovet Narodnogo Khozyaistva	Economic Council
TINRO	Tikhookeanskii Nauchno-Issledovatel'skii Institut Rybnogo Khozyaistva i Okeanografii	Pacific Scientific Research Institute of Fisheries and Oceanography
VNIIPRKh	Vserossiiskii Nauchno-Issledovatel'skii Institut Prudovogo Rybnogo Khozyaistva	All-Russian Scientific Research Institute of Pond Fisheries
VNIORKh	Vsesoyuznyi Nauchno-Issledovatel'skii Institut Ozernogo i Rechnogo Rybnogo Khozyaistva	All-Union Scientific Research Institute of Lake and River Fisheries
VNIRO	Vsesoyuznyi Nauchno-Issledovatel'skii Institut Rybnogo Khozyaistva i Okeanografii	All-Union Scientific Research Institute of Fisheries and Oceanography

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